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# THE PRODUCTION OF NEW HYDRANTHS IN HYDRA BY THE INSERTION OF SMALL GRAFTS

BY

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#### INTRODUCTION

During the winters of 1906-1908, I carried on some experiments in grafting Hydra viridis for the purpose of throwing more light on the factors concerned in regeneration. The work was done at the suggestion of Professor Morgan, whom I sincerely thank for his kind interest and help.

In my first series of experiments, which were done with the ordinary green hydra. I tried to discover what material when grafted would give the necessary stimulus to call forth the development of a new hydranth. It was found by experiment that if a tentacle with a small bit of peristome tissue at its base was inserted into the body of another hydra, the stock would regenerate a whole new hydranth at the place of grafting, the grafted tentacle remaining as one of the new circlet of tenacles. The question arose whether the regeneration was due merely to the presence of foreign tissue of any kind or whether it was initiated by some special kind of material; and if so, what kind of tissue this was. To solve this problem, tissue was taken from different regions of the body of other hydras of the same species.

In my second series of experiments, I endeavored to find out the exact origin of the regenerating material. An excellent opportunity to decide this question was offered by the discovery of Mr. D. D. Whitney that the green color can be entirely removed from Hydra viridis by putting the animals in a .5 per cent glycerine solution and leaving them for about three weeks. These artificial

white hydras form perfect grafts with normal green hydras and the two parts remain distinct in color.

In all my experiments, I used for operation a small watch glass coated over with paraffine on the bottom and filled with spring water. The operations were all done under a binocular microscope which has the advantage of giving considerable enlargement and plenty of working distance. After operation, the animals were kept in watch glasses filled with either spring water or aquarium water, renewed daily.

#### Part I

#### GRAFTS TO PRODUCE A NEW HYDRANTH FROM THE STOCK

The object of this series of experiments was to determine, first, what tissue when grafted into the body of a normal hydra would cause a new head to regenerate in the region of the graft; and, secondly, in what region of the stock the graft must be made in order that the new hydranth develop. To test these questions, material was taken from various regions of the body and grafted at different levels along the bodies of other hydras. All these experiments were done with Hydra viridis as both graft and stock.

#### Group A

# Tentacle with Peristome at Base as Graft

For operation in the present group of experiments, two hydras were put into the paraffine-coated watch glass and one was cut just below the circle of tentacles. This circlet was cut in one radius so that the tentacles extended out from the peristome in a straight line or slight curve. From this line of tentacles I cut off one, being careful to leave some peristome tissue attached at its base. As quickly as possible I made a small transverse slit in the body wall of the other hydra with a small scalpel or sharpened needle, and inserted the prepared tentacle into this slit by means of a needle. If the operation was successful, the raw surfaces healed so that after a few hours there resulted a perfectly normal hydra with the exception of a tentacle projecting from some part

of the body. In this way I inserted a tentacle with a small bit of peristome tissue at its base in different regions of the stock hydras with varying results.

#### Series I Graft Made in Middle of Stock

Result 1. The usual result following the graft of a tentacle with a bit of peristome tissue at its base into the middle region of a hydra was the outgrowth of a new hydranth from the region of graft, the grafted tentacle persisting as one of the new circlet. The result can be best shown by a specific instance.

On February 19, a tentacle with peristome tissue at its base was grafted in the middle of the body of a healthy green hydra. After a few hours, the wounded surfaces had healed and there resulted a hydra perfectly normal except for the protrusion of a tentacle from the middle of its body (Fig. 1). On the next day, a slight outpushing of the body wall around the tentacle was observed. On February 21, two days after operation, this outpushing could be distinctly recognized as a new hydranth. The new hydranth consisted of a short body protruding from the body of the stock, having at its distal end the large grafted tentacle and three very short tentacles (Fig. 2). On the following day these new tentacles were distinctly longer but not so long as the grafted tentacle. On February 23, the fourth day after operation, a small fifth tentacle had appeared and the grafted tentacle was still distinctly larger than the regenerated ones (Fig. 3). The difference in length of the tentacles was gradually lost by the further growth of the regenerated ones until on February 28, the hydra appeared as a double-headed hydra, one head bearing six tentacles and the other, the regenerated head, five; the original head was still considerably longer than the regenerated one (Fig. 4). That this new hydranth was functional and similar in all respects to the old hydranth was proved by the fact that it could capture and ingest food with as great ease as the old one. This double-headed hydra remained in the condition described without further elongation of the new hydranth until it died on March 14. Similar results have been obtained in ten other similar grafts. In some cases, however, the new hydranth grew longer than in the case described, so that it was of about the same length as the original head.

The axial relations assumed by the new head in reference to the common foot were not constant for even the same hydra at different times. The new head was sometimes at right angles to the stock hydra, it sometimes formed a right angle with the foot and a straight line with the original head, it sometimes formed an obtuse angle with the foot making a Y-shaped structure, and it was sometimes at an acute angle with the foot forming a x-shaped figure. The new hydranth never showed any tendency to travel toward the aboral end of the stock hydra. This is of interest in connection with Miss King's experiments in grafting whole heads into the side of stock hydras. As a result of her work, she found that the graft either separated from the stock in from 14 to 22 days, or migrated toward the foot region and separated in from 5 to 7 weeks. These two modes of separation are due, she concludes from her experiments, to the "axial relations assumed by the components of the graft." If the graft remains at right angles to the trunk, separation takes place without migration; if the graft forms a Y-shaped structure with the stock, it migrates toward the aboral end before separating. The new hydranth that is formed in my experiment seems to act quite differently. It has no definite axial relations with the stock and does not migrate toward the foot. As to the final separation of the regenerated hydranth from the old stock, I can say nothing, for I have not succeeded in keeping these grafts more than a month after operation, and they have not separated within that time.

Result 2. In one case grafted February 19, after a slight outpushing of the body wall around the grafted tentacle and the formation of three additional tentacles (Fig. 5), a process of absorption set in. On the seventh day after operation, the projection of the body wall to form a new hydranth was no longer visible, the four tentacles emerging directly from the body of the stock (Fig. 6). Four days later, one of the four tentacles was absorbed, and just below the place where the tentacles were present, a small protrusion on the body wall was noticed (Fig. 7) which on the following day could be definitely determined to be a bud (Fig. 8). The

other three tentacles were absorbed one by one, while the bud was developing, until on March 12, three weeks after grafting, the whole regenerated material had been absorbed and the bud had pinched off leaving the stock as a normal hydra.

The departure from the usual result may be accounted for in this case by the formation of the bud. It may be that the stimulus for regeneration was present and excited the growth of new tissue as is evidenced in the outgrowth of three new tentacles, but when the bud developed, the available material was used for its development, thus depriving the regenerating hydranth of any means of growth.

Result 3. In only two instances, no regeneration was stimulated by the graft of a tentacle with its basal tissue into the side of the stock hydra. But a gradual process of absorption set in, so that after two weeks no trace of the tentacle was left. It is possible that this result was due to the fact that none of the peristome tissue was left at the base of the tentacle, this bit of tissue being accidentally broken off from the tentacle in the process of grafting; or, the results obtained in these two instances may have been an exception to the usual result.

#### Series II Graft Made in Foot of Stock

Result 1. The usual result following grafting a tentacle with a small bit of peristome tissue at its base into, or in the region of, the foot of a normal hydra, was the outgrowth of a diminutive hydra at the point of grafting. The grafted tentacle was partially absorbed as regeneration took place, till it assumed the proportion proper for the small hydra. This small hydra pinched off from the stock in three or four days after grafting, sometimes with only the grafted tentacle present, sometimes with one or two regenerated ones.

Following the history of one of these grafts, we find it as follows: On March 5, I inserted a tentacle with a bit of peristome tissue at its base into the foot of a normal green hydra (Fig. 9). On the next day, there was a very slight outpushing of the body wall of the stock carrying the grafted tentacle with it. On March 7, this

outpushing had increased and two new tentacles were just beginning to grow out from its distal end beside the grafted tentacle (Fig. 10). The grafted tentacle had decreased in size noticeably. This outgrowth was distinctly different from that observed when the tentacle was grafted in the middle of the stock, being much smaller in circumference. On the next day, March 8, the ourgrowth could be clearly recognized as a minute hydranth with two short tentacles and one longer one, the grafted tentacle, which had become still further reduced in size by absorption (Fig. 11). In the afternoon of the same day, this small hydranth pinched off from the stock. On March 11, owing to the further absorption of the grafted tentacle and the further growth of the two regenerated tentacles, this hydra appeared as a typical hydra of very minute size, with three tentacles of about equal length arranged about the hypostome (Fig. 12). The volume of the small hydra was not more than one-tenth that of the stock from which it pinched off. In several other similar experiments, the same history followed except that the small hydra separated with only two tentacles present, the grafted one (reduced in size) and one regenerated one. In these cases, a third tentacle usually appeared after the hydra had pinched off. In still other similar experiments, there were no new regenerated tentacles formed, but the small hydra pinched off with only one tentacle, the grafted one (Fig. 13). One or two tentacles were formed, however, after the small hydra had separated.

Result 2. In two out of about twenty grafts in the foot region, the tentacle grafted was slowly absorbed.

Result 3. In two other cases, abnormal hydras resulted from the graft of a tentacle in the foot region. An outgrowth from the foot carried the grafted tentacle along with it, as though to form a new hydranth (Fig. 14). But the tentacle was absorbed while the outgrowth enlarged, and no separation took place before the end of two weeks when these abnormal hydras died (Fig. 15).

#### Series III Graft Made in Circlet of Tentacles of Stock

The insertion of a tentacle into the circlet of tentacles of a normal hydra in no instance induced the formation of a new hydranth. The tentacle either remained as grafted or instigated the outgrowth

of one or two additional tentacles. In one case two new tentacles grew out two days after grafting a tentacle into the circlet, making ten tentacles in all (Fig. 16). A week later, three of these became fused at the base (Fig. 17). The process of fusion spread until the three tentacles formed a single tentacle, leaving eight in the circlet, one more than the original number. Unless, however, as in this case, an abnormally large number of tentacles was present, no later absorption took place after regeneration.

#### Series IV Graft Made Below Circlet and Above Middle of Stock

Result 1. In the majority of cases, this graft instigated the outgrowth of a new hydranth. The history of these cases is similar to that which followed the graft of a tentacle in the middle of the body. A double-headed hydranth resulted from the graft, but the two heads were considerably shorter in proportion to the body than in the case of the middle-region grafts (Fig. 18).

Result 2. In one case, after a slight outpushing of the body wall and the regeneration of two new tentacles (Fig. 19), this new hydranth coalesced with the old hydranth a week after grafting (Figs. 20, 21). There thus resulted a single-headed hydra with an extra number of tentacles.

Result 3. In another case, no new tentacles were regenerated, but the grafted tentacle was included in the old hydranth by the shifting downward of the other tentacles (Fig. 22).

Result 4. In one case, the grafted tentacle was absorbed slowly so that after two weeks, no trace of the graft was left.

# Series V Graft Made Between Foot and Middle of Stock

Result 1. In most cases, this graft followed the same history as a foot-region graft. A minute hydra developed from the body wall of the stock, and pinched off in three or four days after grafting, when possessing only one or two tentacles (Fig. 23).

Result 2. In several cases this graft gave rise to a new hydranth similar to that which is found in the middle region.

Result 3. In one case, the grafted tentacle itself became a small new hydranth by enlarging; and on this former tentacle small

tentacles grew out (Figs. 24, 25). By further increase in size and the formation of additional tentacles, there resulted a double-headed hydra, one of whose heads was somewhat longer and larger than the other (Fig. 26).

Result 4. In one case, the grafted tentacle was slowly absorbed without any regeneration.

From the foregoing group of experiments (Group A), it is evident that in every region of the body, the graft of a tentacle with a bit of peristome tissue at its base may cause regeneration on the part of the stock. Moreover, the regeneration in every region except in the circlet of tentacles takes the form of a new hydranth, of normal size in the anterior and middle regions of the stock, and of minute size in the posterior and foot regions.

## Group B

Two Tentacles with Peristonie at Base as Graft in Opposite Sides of Stock

In this set of experiments, I endeavored to find out whether a second tentacle with peristome tissue at its base inserted soon after the first in the same region of the body on the opposite side would cause a second new hydranth to regenerate. In some cases the second tentacle was inserted about two hours after the first, and in others it was inserted a day later, but both methods gave practically the same results.

Result 1. In two out of six cases, both grafted tentacles caused the outgrowth of hydranths. One of these was grafted on March 20 (Fig. 27); two days later, there was a slight outpushing of the body wall of the stock at the base of each tentacle, a new tentacle having developed on one of the outgrowths (Fig. 28). On March 25, the hydra had two quite well developed but small hydranths in the middle region, each having two tentacles, a grafted and a regenerated one (Fig. 29). The smaller one of these was gradually absorbed, till on March 30, no trace of it was left, the hydra having only two heads, one with seven tentacles and the other, the regenerated one having four tentacles, one longer grafted one and three

shorter regenerated ones (Fig. 30). By April 1, these four tentacles were of the same length, and by April 5, a fifth tentacle had appeared, so that the hydra was a typical double-headed animal (Fig. 31). In the other similar graft, after the outgrowth of two small hydranths at the base of each grafted tentacle, they migrated from opposite sides so as to be adjacent (Fig. 32). A process of fusion then set in, so that ten days after grafting, the two regenerated hydranths, each with three tentacles, were separate only at their distal ends (Fig. 33). This fusion was complete two days later, resulting in the formation of a double-headed hydra.

Result 2. In two other cases of similar grafts, only one of the grafted tentacles gave rise to a new hydranth, the other one being gradually absorbed (Fig. 34).

Result 3. In the other two cases, both tentacles were absorbed without any regeneration.

From this group of experiments it is evident that each of two tentacles with peristome tissue at their base, when grafted into the middle region of a hydra, may cause the regeneration of a new hydranth. However, a process of fusion or absorption sets in sooner or later, so that only one of the regenerated hydranths remains.

#### Group C

# Tentacle Without Peristome at Base as Graft

Having obtained so definite a response on the part of the stock to a grafted tentacle, I next tried to find out what part of the grafted tentacle was responsible for the regeneration. In this group of experiments I grafted just the tentacle without any peristome tissue at its base into the stock. This operation was somewhat difficult as the raw surface of the tentacle healed over rapidly and would not then adhere to the cut surface of the stock. About eight times, however, by performing the operation very quickly, I was successful. All the experiments gave the same result, whether the graft was made in the middle or foot region of the stock. There was no outpushing of the body wall of the stock, no regeneration whatever, but, on the contrary, the grafted tentacle

was slowly absorbed so that after about ten days no trace whatever of the graft was left.

These experiments show that the graft of just the tentacle without peristome tissue at its base does *not* stimulate the regeneration of a hydranth.

#### Group D

Peristome at Base of Tentacle Without Tentacle as Graft

#### Methods

In one set of these grafts, I cut off a tentacle with the peristome tissue at its base and inserted it into the body of the stock as described under Group A. Then after the raw surfaces of the stock and graft had healed, I cut off the grafted tentacle close to the body wall, thus leaving grafted in the stock the bit of peristome tissue that was at the base of the tentacle. In another set, I cut off a circlet of tentacles, then cut off a few tentacles at their base, close to the circlet, and used a small piece of the remaining ring of peristome tissue. This I grafted quickly into a previously prepared cut in the body wall of the stock. Both methods gave the same result.

#### Series I Graft Made in Middle of Stock

Result 1. In three out of five cases tried, the graft in the middle region of the body gave rise to a new hydranth. The outgrowth was similar to that initiated by the graft of a whole tentacle and peristome tissue at its base. The body wall of the stock at the region of graft pushed out and new tentacles were formed on it (Figs. 35-37).

Result 2. In two other similar experiments, absorption took place and no regeneration occurred.

#### Series II Graft Made in Foot Region of Stock

Result 1. In two out of three cases in which the peristome tissue at the base of the tentacle was grafted into the foot region of the

stock, there grew out a minute hydra, similar in formation and appearance to that instigated by a graft of a tentacle and basal tissue into the foot (Figs. 38, 39).

Result 2. In the other case, the tissue was absorbed and no regeneration took place.

#### CONCLUSION

This group of experiments shows that the whole tentacle is not necessary for the production of a new hydranth on the part of the stock. But merely the peristome tissue at the base of the tentacle is sufficient, when grafted, to instigate the outgrowth of a new hydranth.

#### Group E

# Tissue Anterior to Circlet of Tentacles as Graft

The amount of tissue anterior to the circlet of tentacles is very small and it was found very difficult to cut it off without getting into the region of the tentacles. By waiting, however, until a large hydra was fully expanded and very quickly bringing the scalpel just anterior to the tentacles, I found it possible in five cases to get this small bit of tissue cut off. This was grafted into the middle region of the stock.

In none of the five cases was any regeneration instigated. The grafted tissue was absorbed, so that the day after grafting no trace of the graft was to be seen.

The tissue anterior to the circlet of tentacles will not give the stimulus necessary for the outgrowth of a new hydranth, when grafted into the body wall of a stock hydra.

## Group F

# Tissue From other Regions of the Body as Graft

In this set of experiments, I endeavored to find out whether tissue from any region of the body other than at the base of the tentacle, would, when grafted, give rise to a new hydranth. For this purpose, I cut off a small ring of tissue from various regions of the body, including the region just beneath the circlet of tentacles and grafted it into the middle region of a hydra. These rings in no case gave rise to a new hydranth, but were soon absorbed.

If, however, instead of a small ring of tissue, a large ring was grafted, the result was different. The experiments were done in the following manner: A hydra was cut in two beneath the circlet of tentacles; the foot was then cut off from the lower part. The aboral end of this band of tissue was then grafted into the side of a normal hydra. On the following day, the graft had developed tentacles. In this case, however, it must be noted that the regeneration is entirely on the part of the graft and not of the stock, and the stock takes no part in the formation of new tissue.

The conclusions drawn from these experiments is that no other tissue than that at the base of the tentacles is capable of so stimulating the stock as to cause it to produce from its body wall a new hydranth.

## Group G

# Regenerating Tissue as Graft

This series of experiments was undertaken for the purpose of finding out whether tissue which has begun, in the process of regeneration, to be differentiated into tentacle-forming material, would, when grafted, influence the body wall of the stock to regenerate a new hydranth. The method adopted was as follows: A green hydra was cut in two at about the middle of the body. The posterior half was ther left till the following day when the wound had healed and the process of regenerating a new hydranth had started, although no tentacles had formed. A very small piece was cut off from the oral surface, and this was grafted into the side of a normal hydra. On the next day an outpushing of the body wall had occurred and a day later two tentacles had formed on the new hydranth (Figs. 40, 41). By leaving the regenerating piece different lengths of time before grafting part of it into the stock, it was found that about ten hours was the minimum that would give regeneration. If left only seven hours before grafting, the graft

was absorbed and no regeneration took place. The length of time necessary for regeneration to be far enough along for a piece of the regenerating tissue to call forth a hydranth from the stock when grafted, would of course depend on the rate of regeneration from an exposed surface, and this has been found to vary in different regions of the body. If, therefore, the original cut were made just below the tentacles instead of in the middle of the body, less than ten hours would probably suffice for the regenerating tissue when grafted to call forth a new hydranth from the stock.

## Group H

## Tissue of Bud as Graft

In this experiment, a small piece of the anterior end of a young bud whose tentacles had not yet been formed, was cut off and grafted into the middle of the body of a stock hydra. There followed as a result of the graft an outgrowth of a new hydranth similar to that instigated by the insertion of regenerating tissue into the stock (Figs. 40, 41). That this outgrowth was a new hydranth and not an ordinary bud is shown by the length of its tentacles. These were long like those of a hydranth and not short like those of a bud (cf. Figs. 41 and 8.)

#### General Conclusions to Part 1

The conclusions to be drawn from the whole set of experiments are as follows. A new hydranth can be formed by a hydra in any part of its body except in the tentacle region, when the necessary stimulation is given by a grafted piece. The stimulus can be given by no other grafted piece than the material that lies at the base of the free tentacles, or that lies in a regenerating hydranth or a bud. The transformation of body wall material into hydranth material depends therefore not on the size of the piece grafted into it, but entirely on the differentiation of the grafted material. If the material grafted has been entirely differentiated into material lying at the base of a tentacle, or if it has been sufficiently differentiated by regeneration or budding into this kind of material, this

tissue will cause the body wall tissue of a normal hydra to change its differentiation and function and become tentacle and hypostome tissue.

#### PART II

#### ORIGIN OF REGENERATING TISSUE AND FATE OF ABSORBED TISSUE

In the second series of experiments, I tried to find out the source of the regenerating material and the fate of absorbed material in the foregoing and other experiments. Attempts have been made to solve these questions in some cases by grafting Hydra fusca and Hydra viridis, a brown and a green hydra, but these attempts have proved unsuccessful, for the two species do not graft well. Although the graft has been made to stick for a day or so, it always pulls away from the stock before any results can be obtained. Miss King has attempted to solve the question in her experiments by using light and dark green individuals of Hydra viridis. She states that she is able to distinguish the two shades for two or three weeks, at the end of which time they fuse. In combinations between the artificial white hydras produced by Whitney's method and the normal green one the contrast between the tissue of the stock and that of the graft is very distinct and remains so for about a month.

## Group A

# Regeneration of Hydranth

In order to determine the exact source of the material forming the new hydranth in the preceding set of experiments (Part I), the following experiments were done.

# Series I White Tentacle with Base Grafted in Middle of Green • Hydra

The result of this grait was in six cases the outgrowth of green tissue from the stock carrying the white tentacle with it, and the later regeneration of green tentacles (Fig. 42). The new hydranth material, then, must come from the body wall of the stock, while the grafted tentacle remains as one of the new circlet.

#### Series II White Tentacle with Base Grafted in Foot of Green Hydra

The result of this graft was in four cases the outgrowth of a small amount of green tissue at the base of the white tentacle to form a minute hydranth (Fig. 43). This pinched off as a small green hydra with one white tentacle (Fig. 49). Four days after operation a second tentacle appeared on it, composed of green tissue; some of the white material of the grafted tentacle seemed to have been absorbed into the anterior part of its body (Fig. 50).

From these experiments the conclusion must be drawn that it is principally the material of the body wall of the stock and not the hydranth material of the graft that forms the new hydranth. The ectoderm and endoderm cells that have been body wall cells are therefore changed over into cells composing tentacles and hypostome.

## Group B

## Absorption

In order to find out the fate of the material grafted into the stock hydra when no regeneration took place; whether the material was incorporated into the tissue of the stock hydra, or whether it was so absorbed that it no longer existed as such, the following experiments were performed.

#### Series I Green Tentacle Without Base Grafted into White Hydra

As the green tentacle was absorbed, the green material spread along the body wall of the white hydra for a small area at the union of stock and graft (Figs. 44, 51). The two hydras on which the experiment was performed were unfortunately lost before complete absorption. But it was evident that the green tentacle material was being transformed into body wall material and incorporated in the stock.

#### Series II Green Circlet in White Hydra

After the graft of a green circlet of tissue from a normal hydra into the middle region of the body of a white hydra, this tissue remained in the white body as a patch of green (Fig. 45).

We conclude then, that when a piece of tissue is grafted into a normal hydra and does not cause regeneration, the grafted tissue is incorporated into the body wall of the stock. The cells that have formed tentacle or body wall tissue are made over in the stock into body wall cells. The tissue is absorbed, not in the sense that it disappears, but in the sense that it becomes one with the tissue of the stock.

# Group C

# Graft's of White and Green Hydranths

A few experiments were performed with green and white hydras to discover if possible whether a hydranth that was grafted in the side of another hydra kept its individuality or whether the tissues of the two hydras fused. In one of the two successful grafts in which a short green head was grafted into a white hydra at about the middle region, the graft retained its individuality and was of approximately the same size and in about the same position at the end of two weeks as at the time of graft (Fig. 52). As the hydra did not live until the graft pinched off, the final result was undecided, but it seemed probable that it would pinch off at the line of union of graft and stock. The second graft was more interesting for the reason that the green grafted head increased in size until of equal length with the head of the stock and then migrated down toward the foot end of the white hydra (Fig. 46). The point to be noted is that in increase in size, the new material came not from the larger white hydra but was formed by the green hydranth. The graft not only kept its individuality but also completed itself by regenerating new material. A third experiment of somewhat different kind shows the same principle. A white and green head were grafted together by their aboral ends, the green one being somewhat shorter than the white one (Fig. 53). Both hydranths kept their individuality and the green one regenerated new tissue so as to become of equal length with the white one (Fig. 54). In this condition, the graft died, evidently just before separation would have taken place.

From these experiments we conclude that the grafted hydranth, although intimately associated with the stock, keeps its individual-

ity. This conclusion agrees with that of Miss King who, in similar experiments used hydras of different shades of green as stock and graft.

## Group D

# Graft of Green Foot in White Hydra

In four out of five cases in which the lower half of a hydra was grafted by its cut oral surface into the middle region of a normal hydra, the grafted foot was absorbed. The history of the other case was as follows: On February 22, a green hydra was cut in two, and the posterior half was grafted by its oral surface into the middle of the body of a white hydra. On February 25, the graft had moved down somewhat toward the foot of the stock (Fig. 55). On February 27 a new head was evidently being formed at the union of graft and stock, of graft material (Fig. 56). On February 28 the graft had quite completed itself, having formed several tentacles, but was still attached near the foot region to the stock (Fig. 47). On February 29, the green hydranth including part of the grafted foot and the regenerated head separated off from the white hydra, leaving, however, a small amount of green foot tissue attached to the stock (Fig. 57).

From these experiments I should conclude that if the graft asserts itself sufficiently not to be absorbed by the stock, it maintains its individuality, forms its own tentacles and separates off as a complete hydra.

#### Group E

#### Reversed Polarity in Green and White Grafts

Cases of heteromorphosis in hydra produced by grafting have been reported by Wetzel, Peebles, and King. A heteromorphic foot has been produced by Wetzel by removing the foot ends of the two hydras, grafting the two aboral ends together and cutting off one head close to the tentacles. A normal hydra was produced with a foot at the former oral end. Peebles performed the reverse experiment, cutting off the heads of two hydras, grafting them together by their oral surfaces and subsequently cutting off one

foot close to the line of union. Heteromorphic heads were produced in five cases on the exposed aboral surface. King succeeded in obtaining heteromorphic structures by cutting off both ends of a head-to-head or a foot-to-foot graft close to the line of union, leaving a ring of tissue with two aboral or two oral ends exposed. In most cases, she found that normal hydras resulted, one end having reversed its polarity so that a head was produced from the exposed aboral surface or a foot from the exposed oral surface. It has never been definitely shown whether these heteromorphic structures are really formed from material whose polarity has been reversed through the influence of the complementary structure or whether the material has rearranged itself so that polarity is not really reversed.

That such a rearrangement is possible is shown by an experiment in which I cut a hydra longitudinally and reversed the two halves so that each free end consisted of half foot and half head with tentacles (Fig. 58). Two days later it was evident that the foot material of each end was migrating from its position near the tentacles to the middle of the body (Fig. 59). That this structure was produced by a migration of the foot material and not merely by a split along the line of graft, separating the original half head and half foot, is shown by the fact that the free foot was not of equal length with the hydranth, but was only a small projection from the surface. The final result of this graft was the separation of the two original half-hydras into two complete hydras (Figs. 60, 61). In this case, then, there has been a migration and rearrangement of material. Another instance of migration is the experiment described in Group C, where the grafted hydranth moved down along the stock (Fig. 46) from the middle to the foot region. Many similar cases of migration have been described by King and Rand and Hefferan.

Is it not possible that in the case of the supposed heteromorphic heads, the aboral material of the graft has wandered in, leaving exposed the oral material of either stock or graft, so that the head really develops not from an aboral layer of tissue but from the oral layer?

The answer to this question has been definitely determined, I

think, by the following experiments: A white and a green hydra were both cut a little beneath the tentacles and the two cut oral surfaces grafted together. A few hours later, after the graft had become secure, the green portion was cut off leaving a circle of green tissue with aboral end exposed and attached by its oral end to the oral surface of the headless white hydra (Fig. 62). In three cases, tentacles appeared at the free aboral end and these tentacles were formed of green material, the oral material of the white stock having no part in their composition (Fig. 63). In a few other cases, one or two of the tentacles were formed of white material, the rest of green (Fig. 64). Whether this result was due to a somewhat oblique cut, so that the circle of green was minimal in one place, or whether the material rearranged itself, some of the green migrating posteriorly and the white anteriorly was not determined. In the former cases, where all the tentacles were green, there was no migration of the white oral material. These experiments did not show, however, that there might not have been a migration of material in the green tissue itself, the oral material going anteriorly and the aboral posteriorly. In one experiment, it was conclusively shown that such migration did not take place. In this case tentacles formed not only on the exposed green surface, but also at the union of graft and stock (Fig. 48). Moreover, both sets of tentacles were composed entirely of green material. One set must therefore have come from the oral material which still remained at the junction of graft and stock, and the other set from the aboral material which lay at the exposed surface. This was an undoubted case of heteromorphosis in the strictest sense of the word, and throws some light on the meaning of reversal of polarity in connection with heteromorphic structures. We find in this experiment, that although polarity has been reversed in so far as normal foot-producing material becomes head-forming material under the influence of the larger piece, the original polarity which determined that the head-forming material should be headforming has not been lost. It would seem that the original polarity of the reversed piece is not altered, but that on account of the relation of the graft to the stock, a secondary polarity has been assumed. This secondary polarity is that of the stock which

asserts itself in the new material which now becomes a part of its own body. If this secondary polarity be conceived as preponderating over the primary polarity of the graft, this original polarity would be entirely submerged, and this is probably the case in the former experiments where only heteromorphic tentacles were formed. On the other hand if the primary polarity preponderates over the secondary we should have tentacles at the junction of graft and stock and no heteromorphic structure. Many cases of this result have been reported by Peebles and King.

In three cases I succeeded in obtaining heteromorphic feet by grafting the two cut aboral ends of a white and a green hydra and then cutting off the head of the green end leaving an exposed oral surface of green tissue (Fig. 65). This graft resulted in the formation of a normal hydra consisting of a head end of white material and a foot end of green material (Fig. 66). The foot end consisted of a true foot as evidenced by the presence of the characteristic sticky secretion which made the foot adhere to the substratum. These were evidently cases of heteromorphosis, the polarity of the stock so influencing the graft as to preponderate over its original polarity, thus calling forth a foot from its exposed oral surface. The production of heteromorphic feet in these experiments confirms the like experiment of Wetzel and is opposed to the results of Peebles who obtained no heteromorphic foot in the fifteen cases tried. No hook-like processes such as described by Wetzel as preceding the formation of a heteromorphic foot appeared in my experiments.

#### CONCLUSIONS

As a result of the experiments recorded in this paper, the following conclusions may be drawn:

1. The fate of a graft in Hydra viridis depends on several factors. Rand states that "the fate of the graft depends upon its degree of specialization." King states that "the fate of a graft depends, not upon its degree of specialization, but primarily on its size and to some extent on its position in the stock." From my experiments, I should conclude that the fate of a graft depends (1) primarily on its specialization. If the tissue grafted is a smal

amount of body wall tissue, or pure tentacle tissue, or pure hypostome tissue (tissue anterior to the tentacles) or a small amount of foot tissue, it is absorbed. If it is tissue lying at the base of the tentacle, whether it includes a tentacle or not, it becomes part of a new hydranth which under its stimulation grows out from the stock. The fate of a graft depends (2) on the size of the piece grafted. A large piece of body wall tissue with the oral end exposed in a lateral graft gives rise to a new hydranth, a small piece is absorbed. A large piece from the foot may produce a new hydranth when grafted laterally, a small piece is absorbed. The fate of a graft depends (3) on the position it occupies in the stock. If in the circlet of tentacles, a new hydranth is not produced by the graft of a tentacle with peristome tissue at its base, but in any other region of the body a new hydranth may be produced. In the foot region this hydranth is very minute; in the middle region it is of normal size. The fate of a graft depends (4) on its polarity. If a band of tissue is grafted by its aboral end to the oral surface of a halfhydra, leaving the oral surface of the graft exposed, a normal hydra is produced, the tentacles growing on the exposed oral surface. If a band of tissue is reversed and grafted by its oral end to the oral surface of a half-hydra, leaving exposed the aboral surface of the graft, unless sufficiently small, tentacles grow out at the line of union of the two components, and not at the free end.

- 2. No matter how specialized a tissue has become, it can, when grafted, be made over into a different kind of tissue and be incorporated into the body of the stock. For example, the material that had become differentiated into tentacle tissue can lose its differentiation and become body wall tissue and function as such. Likewise, differentiated tissue of the stock can, under the influence of special grafts, be made over into other kinds of differentiated tissue. For example, the foot tissue can, under the influence of a grafted tentacle with basal tissue attached, be transformed into the body wall and tentacle tissue of a new hydranth.
- 3. A grafted hydranth and a grafted foot, when not absorbed, keep their individuality and do not become one with the stock.
- 4. A new hydranth can be stimulated to grow out from a hydra by (1) the graft of the peristometissue at the base of the tentacle, with

or without the tentacle itself, and by (2) the graft of the material of a regenerating hydranth and by (3) the graft of the material of a bud. Neither a wound nor the graft of any other kind of tissue will stimulate the stock to send out a new hydranth.

5. A reversal of polarity may take place in a graft, resulting in the production of a heteromorphic structure, if the polarity of the stock preponderates over that of the graft.

Zoological Laboratory Columbia University

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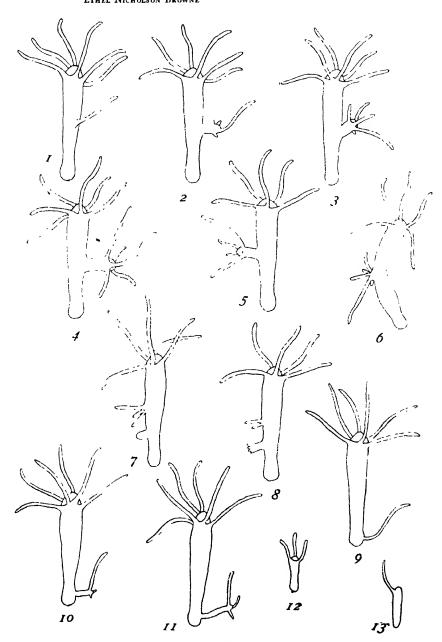
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#### PLATE I

Figs. 1-5 Graft of tentacle with peristome tissue in middle of stock.

Figs. 6-8 Absorption of new hydranth.

Figs. 9-13 Graft of tentacle with peristome tissue in foot of stock.



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#### PLATE II

Figs. 14-15 Abnormal hydra produced by foot-region graft.

Figs. 16-17 Graft of tentacle with peristome tissue in circlet of tentacles.

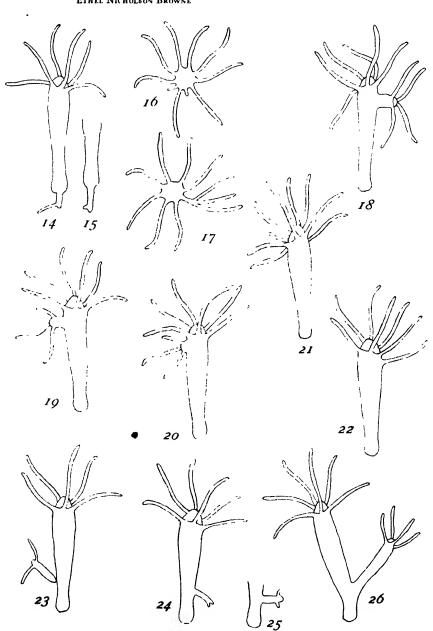
Fig. 18 Graft of tentacle with peristome tissue below circlet and above middle

Figs. 19-21 Fusion of old and new hydranths.

Fig. 22 Grafted tentacle included in old circlet.

Fig. 23 Graft of tentacle with peristome tissue between foot and middle.

Figs. 24 -26 Transformation of grafted tentacle into new hydrauth.



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#### PLATE III

Figs. 27-29 Graft of two tentacles with peristome tissue in opposite sides of stock.

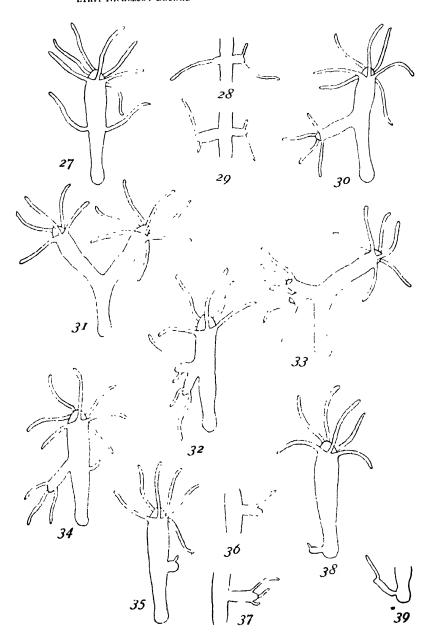
Figs. 30-31 Absorption of one new hydranth, and growth of other.

Figs. 32-33 Migration and fusion of two new hydranths.

Fig. 34 Absorption of one grafted tentacle.

Figs. 35-37 Graft of peristome tissue without tentacle in middle region.

Figs. 38-39 Graft of peristome tissue in foot.



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#### PLATE IV

Figs. 40-41. Graft of regenerating tissue.

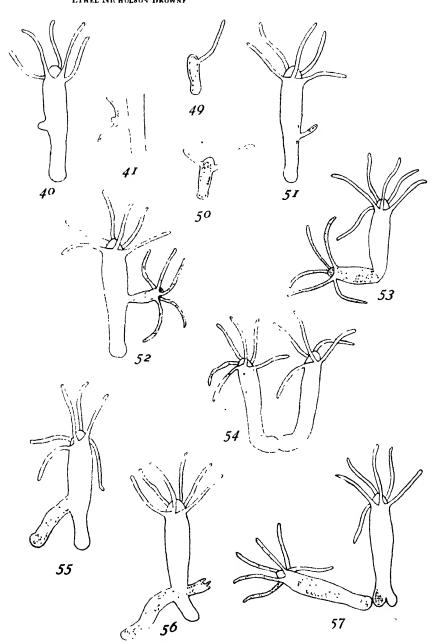
Figs. 49-50 Small hydra produced by graft of white tentacle in foot of green hydra

Fig. 51 Graft of green tentacle without peristome in white hydra

Fig. 52 Graft of green hydranth in white hydra.

Figs. 53-54 Graft of green and white heads.

Figs. 55-57 Graft of green foot in white hydra.



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#### PLATE V

- Fig. 42 Graft of white tentacle with peristome in middle of green hydra.
- Fig. 43 Graft of white tentacle with peristome in foot of green hydra.
- Fig. 44 Graft of green tentacle without peristome in white hydra.
- Fig. 45 Graft of green body tissue in white hydra.
- Fig. 46 Graft of green hydranth in white hydra.
- Fig. 47 Graft of green foot in white hydra.
- Fig. 48 Heteromorphosis in reversed ring of green tissue grafted on white stock.

ETHEL NICHOLSON BROWNE



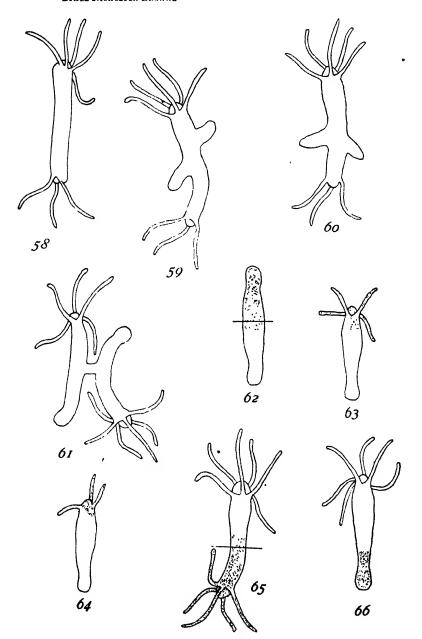
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#### PLATE VI

Figs. 58-61 Graft of reversed halves of a longitudinally split hydra Figs. 62-64 Heteromorphic heads in green and white graft Figs. 65-66 Heteromorphic foot in green and white graft

#### ETHEL NICHOLSON BROWNE



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# THE EFFECT OF THE DESTRUCTION OF PERIPHERAL AREAS ON THE DIFFERENTIATION OF THE NEUROBLASTS.

#### M. L. SHOREY

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#### I INTRODUCTION

Observation of surgical cases in which the amputation of a limb has been followed by the degeneration of the nerve fibers innervating it, and finally of the nerve cells from which they arise, and cases of paralysis accompanied by the atrophy of the muscles

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concerned, have demonstrated that an intimate relation exists between the life of a muscle and that of its motor nerves. least since 1851, when Weber described an abnormal calf embryo with the nervous system entirely wanting below the second dorsal vertebra and an accompanying loss of the corresponding muscles, there has been a tendency to believe that there is likewise a dependence in development. Other teratological cases in which a defect in the nervous system has been accompanied by a defect in the organs normally innervated by the missing nerves have been recorded, and the effect of the absence of the nerves in experiments in regeneration has been adduced as evidence in favor of such a view. It is obvious, however, that neither of these conditions can furnish conclusive evidence regarding developmental processes, for, in the case of monstrosities it is impossible to know the nature of the original lesion, and in regeneration, while there are doubtless similarities between it and normal development, it is evident that neither the conditions nor the processes can be iden-

Bardeen, in 1900, made careful observations on the relations between the myotomes and nerves at early stages of embryonic development, and from the fact that there is no apparent contact between the two until a considerable degree of differentiation has been reached concludes that early development is independent. To quote, "The early development of the nerves is one of passive independence, without any immediate relations to the myotomes." But it seems to me that such evidence must be regarded as inconclusive, for the fact that two organs are not in direct contact does not exclude the possibility that the one may influence the other.

To reach decisive conclusions regarding the interdependence of two organs or tissues in development it is necessary to study the behavior of one in the absence of all possible effects from the other. This can be done only by destroying some portions of the developing organism, or by removing the part to be studied to a controlled environment. Both these methods have been used in studying the relation of the nervous system to peripheral organs, but in most previous studies the object of the investigation was to discover the effect of the destruction of nervous tissue on the organ inner-

vated. A few experiments dealing with the effect of an abnormal environment on the developing nerve have, however, been made.

Harrison ('06), experimenting on frog larvæ for the purpose of ascertaining whether other cells than those of the nerve center enter into the formation of the nerve fiber, obtained results which bear on the question under consideration. The spinal cord of these embryos was extirpated, and small pieces of it transplanted under the skin of the abdominal walls. Small nerve trunks arise from these and run for some distance in various directions. portions of the ganglionic crest thus transplanted small ganglia 'and nerves may arise. His own conclusions are as follows: "The nerve center (ganglion cells) is shown to be the one necessary factor in the formation of the peripheral nerve. When it is transplanted to abnormal positions in the body of the embryo it then gives rise to nerves which may follow paths where normally no nerves run, and likewise when the tissues surrounding the center are changed entirely nerves proceeding from that center may develop as normally." In another experiment fragments of the medullary tube, isolated before visible differentiation, were placed in a drop of lymph, and it was found that these develop short fibers. From this experiment he concludes that the outgrowth of the fiber is largely independent of external stimuli, although its direction is doubtless influenced by a number of factors. That the direction is influenced is shown by a number of experiments in which a limb bud transplanted to some other region of the body receives normal innervation from the nerves of the region to which it is transplanted.

Braus ('06) removed one of the anterior limb buds of several toad larvæ, and ten days later found that the brachial plexus was as well developed on the injured as on the uninjured side, although by far the greater part of the musculature had been destroyed, and at the time of the operation no nerve fibers running to the limbs were visible. Older operated embryos showed the brachial plexus present but diminished in size. From these experiments Braus concludes that "Der Befund am Plexus brachialis ist eine sehr deutliche Illustration dafür, dass die Entwicklung der peripheren Nervenfasern unabhängig ist von den Endorganen."

The work of the two last investigators has been published since, or about the time that I began to investigate the relation of nerve and muscle tissue in the chick embryo, and did not reach my hands until a later date. The experiments to be described were undertaken for the purpose of studying the behavior of the developing nervous system, when it is itself left quite intact and with all its relations normal except that a peripheral area to which nerves should be distributed is destroyed.

The work was begun at the suggestion of Professor Frank R. Lillie, and I wish to express my indebtedness to him for proposing a problem which has proved to be of intense interest, and for suggesting the first methods by which it was to be attacked. I wish also to express my appreciation of his continued interest and helpful criticism. Acknowledgments are also due to Professors C. O. Whitman, C. M. Child, and W. L. Tower for suggestions in regard to illustrations, photography and material, and to Miss Sabella Randolph, Mr. Kenkichi Hayashi and Mr. C. E. Brues for aid in the preparation of drawings and photographs. Figures 36 and 37 are copied from Lillie's Embryology of the Chick.

#### II EXPERIMENTS ON THE CHICK

# A Choice of Material

The chick embryo was chosen as furnishing a form favorable for these experiments; the work of Lillie ('04) in destroying various portions of the embryo had shown that no regulation or regeneration was to be expected and my own work has entirely confirmed these results. After two days of incubation, the earliest period at which I have operated, and in regions where no differentiation is apparent, the primordium for each organ is so definitely laid down that the destruction of it, or any considerable portion of it, results in a corresponding loss in the fully formed animal. Lillie's work had also shown that comparatively large areas may be destroyed without interfering with the normal development of the rest. It would therefore be possible to destroy the primordia of definite muscles, or of sensory areas, before they had been penetrated by nerve fibers, and then determine the course of the nerves which

should innervate them. The rapidity of development, and the availability of material at practically all times of the year were also points in favor of the chick.

#### B Character of the Operation

It was decided that the first experiments should be the removal of the wing and after some preliminary tests the period at which the wing bud is just apparent to the naked eye as a small elevation on the body wall was chosen as the most advantageous for the operation. The period of incubation before this stage is reached varies from 68 to 88 hours according to the time of year, the development of the embryo when the egg is laid, and fluctuations in the temperature of the incubator, so that it is impossible to tell in exactly what condition the embryo will be found when the egg is opened, but Fig. 1, a photograph taken in this case after 74 hours of incubation, gives approximately the external appearance at the time when most of these operations were performed. The wing, was never larger than this, and in the majority of cases it was smaller. 2, a photograph of a cross-section in the region of the wing, shows the relation and development of different parts at this time. Even if the wing bud is removed close to the body, and no part of the wing proper develops, some of the muscles, or portions of them, extending dorsally from it to the vertebræ, and ventrally to the sternum, always appear. It, therefore, seems evident that the primordium for these has not yet grown into the wing, but is still contained in the muscle plate. Parts of the bones of the shoulder girdle will also form, so that only a portion of their primordium can be located in the wing bud at this period.

Lillie has determined that the wing develops from the 17th, 18th, and 19th somites, and that it, with the other structures arising from these somites, is innervated by the 14th, 15th, and 16th nerves, the sensory root arising from the 12th, 13th and 14th ganglia. Later in development these three nerve trunks unite to form a plexus before their distribution to the muscles and sensory areas of the arm, but at this stage they have not yet reached the place of union and each appears as a distinct trunk extending toward the base of the wing but not yet reaching it. Neither is it in contact with the myotome.

The neural tube exhibits but little differentiation; no definite cell areas are marked off within it, and only a thin layer of nerve fibers or axones has formed at the periphery. There is a small ventral root, but the cells from which these axones arise have not yet become localized as the ventral horn.

By operating at this stage of development it was possible to remove the greater part of the wing without any direct injury to the nervous system. As stated above, some structures, or parts of them, belonging to the wing may still develop, and the intrinsic muscles of the vertebral column are entirely uninjured; still the peripheral area, both sensory and motor, is much decreased, and as the greater part of the peripheral nervous system is still to be developed, and most of the differentiation of the spinal cord still to occur, it is possible to study the effect of the loss of the end organ on the size and distribution of the peripheral nerves, as well as its effect on the cells of the central system with which they are connected, either directly or indirectly.

#### C Methods

During the period of incubation preceding the operation the eggs were turned every 12 or 24 hours to prevent the blastoderm from adhering to the shell membrane, but to insure the accurate location of the embryo a number of hours must elapse between the last turning and the operation. The method of opening and closing the egg was essentially the same as that described by Lillie ('03) and earlier by Miss Peebles ('98), but instead of placing strips of egg membrane about the edge of the shell used as a cover for the opening in the operated egg I found it more effective to leave a margin of membrane attached to this cover. If then the curvature of the cover is the same as that of the egg to which it is applied, and there is no break in the membrane, a perfect closure is effected.

Since the embryo lies on the left side (I found only two or three exceptions to this in the fifteen hundred or two thousand eggs used in these experiments) the right wing was always the one removed. For this purpose small spear-headed scalpels, were first used and this method may be used with success if the wing bud has attained considerable size, but for the earlier stages, at which it was finally decided to operate, destruction by electrolysis is much more satisfactory. Number 12 sewing needles, one of them bent so as to penetrate the amnion and lie along the whole extent of the wing bud with as little tearing of the membrane as possible, were used for the operation. These were held in electric handles connected with dry battery cells furnishing a current of about two volts. The destruction is almost instantaneous, so that the current need be applied for but a very brief period.

There existed the possibility that by this method of operation the electric current might penetrate beyond the region with which the needle was in contact, and thus lead to the direct destruction of cells within the spinal cord. Such a possibility is eliminated by the following facts:—in a number of embryos in which the wing was removed by scalpels the same defects appear as in those of the same age operated on by electricity. Moreover the defects always appear in the same region, and follow the same order, and it would scarcely be possible to suppose that an electric current, free to extend in any direction, should always follow exactly the same path and destroy the same cells, leaving those immediately surrounding them quite normal. And a third reason is found in the fact that until twenty-four hours after the operation the path of the current is well defined by means of the presence of the injured tissue, and in specimens killed at this period, and in which it is possible to determine by this means that the injury did not extend into or even close to the spinal cord, the nervous system is still defective and the defects are always of the same nature.

I had at first many infected eggs, and occasional ones later, but as I have at other times operated every day for months with only one or two cases of infection, I believe that only sufficient care is necessary to avoid it entirely. The surface of the egg was disinfected with mercuric chloride, and all instruments sterilized. There continued, however, to be a large percentage of loss from tears in the blastoderm, puncturing of bloodvessels, extensive injury to the amnion, and other causes whose nature was not determined. Probably most cases of death within a short time after the operation are traceable to injury to the circulatory sys-

tem, but when it occurs 5 or 6 days later, as it did in some experiments which I attempted to carry beyond that period, it seems to be in some way connected with defects in the amnion, for in all these cases which were examined carefully an aperture was found in the region of the operation. This would agree with the work of other observers who have shown that anamniote embryos may develop normally for a time, but that later this defect is fatal. That the amnion can, however, regenerate if not too extensively injured and form a completely closed sac was demonstrated by other experiments in which I knew it to have been injured by the operation, so that an improved technique would doubtless obviate this difficulty.

Killing and fixing in sublimate æctic, and staining with Delafield's hæmatoxylin and Orange G was found to be entirely satisfactory for the end desired, and was the method used in the majority of cases.

As the effects would naturally be most pronounced after a considerable interval of time I began with a study of the oldest embryos, those killed between 5 and 6 days after the operation. When the conditions in these had been ascertained, specimens killed respectively 4 days, 3 days, 2 days, and I day after the injury was made were examined, and the results will be given in this order.

Experiments on Embryos After Three Days of Incubation

Specimens Preserved Five or Six Days After the Operation

Experiment 538. This egg was incubated 84 hours and was approximately in the stage shown in Fig. 1 at the time of the operation. The right wing was removed and the egg returned to the incubator for 5 days 5 hours, so that when preserved the age of the embryo was 8 days 19 hours. The only evidence of the right wing was a small rounded elevation scarcely noticeable on a casual examination. The wound was completely healed and the embryo vigorous and entirely normal externally except for the missing limb.

It was killed, sectioned, and stained as given above, and the operated and unoperated sides carefully compared.

The bones and muscles were well defined and easily identified and the course of the nerves traced without difficulty. Fig. 3 shows the location of the chief wing muscles and Fig. 33, A, the peripheral distribution of the brachial plexus on the normal side. Since the branches of the 14th, 15th and 16th nerves which innervate the vertebral musculature are not affected, these muscles being quite intact, and the branches arising from the separate trunks before the formation of the brachial plexus, their distribution has been neglected.

On the operated side the bones of the shoulder girdle are present but defective; the clavicle and scapula are, however, only slightly abnormal. Of the muscles the trapezius, rhomboideus, and subclavius are not noticeably affected; the pectoralis major, pectoralis secundus, coraco-brachialis, latissimus dorsi, teres et infraspinatus and subscapularis are present but defective, while the deltoides supraspinatus, biceps, pectoralis tertius, coraco-humeralis, scapulo-humeralis, and triceps are entirely wanting. Fig. 33, B, shows the peripheral distribution of the brachial plexus on this side. It will be seen by comparing this with A that wherever a muscle is missing the corresponding nerve is also missing; otherwise the distribution is the same as that of the opposite side.

As A and B are drawn on the same scale they show something of the quantitative loss, but measurements give this more exactly. It is difficult to arrive at the exact dimensions of the nerve trunks from sections without making reconstructions, but as a means of comparison each of the three nerves involved was measured directly below the union of the ventral root with the ganglionic root, and the number of sections in which each appears at this place counted.

From the data thus obtained the following percentages of loss for the nerve trunks were calculated:

	WIDTH	THICKNESS
	PER CENT	PER (ENT
14th nerve	34.1	25
15th nerve	17 . 6	36.3
16th nerve	. 35.8	30

Measurements of the ventral roots show this loss:

*	WIDTH	THICKNESS
	PER CENT	PER CENT
14th nerve	31.9	45-4
15th nerve	8.1	33.3
16th nerve		33 · 3

Measurements of the dorsal roots, which are more difficult to take accurately, because in some sections the boundary between them and the vertebræ is obscure, show a less, but still a constant loss:

	WIDTH	THICKNESS	
	PER CENT	PER CENT	
14th nerve	36	۰	
15th nerve	1	21	
16th nerve	38	11	

The ganglia of the operated side were also smaller, and this loss is expressed quantitatively in the following table:

(In this it must be remembered that the 12th, 13th and 14th ganglia correspond to the 14th, 15th and 16th nerves.)

LATERA	AL	DORSO-VENTRAI		
DIAMET	DIAMETER		THICKNESS	
PER CE	NT	PER CENT	PER CENT	
12th_ganglion 24.	. 3	16.7	20	
13th ganglion 22.	- 7	22.7	33 1	
14th ganglion	3	30.2	20	

The loss in the spinal cord will be best shown by a photograph of a typical section (Fig. 14). This was taken in the region of the 15th nerve. The most evident difference here is in the ventral horn, this being decidedly smaller throughout the operated region. It is also noticeable that the abnormality is practically confined to the region of the 14th, 15th and 16th nerves, the first observable difference appearing three sections anterior to the beginning of ganglion 12, and although very careful measurements show slight differences to the eleventh section beyond the 14th ganglion it is markedly less immediately beyond that ganglion. This loss in the ventral horn might conceivably be due to either of two reasons: the number of cells might be less, or the individual cells or part of them, might be smaller, or the loss might be due in part to both of these causes. Two methods of deciding this

question were adopted. First the cells of the antero-lateral part of the ventral horn, this being the region affected, beginning with the section on which the 12th ganglion appears, and going to the section on which the 15th appears, were counted on each side. There is evidently much chance for error in this, particularly as the sections were not cut with this in view, and a change of focus brought different cells into the visual field, but the cells of each section were always counted a number of times, and the probability of error is equally great for each side. While, therefore, the figures obtained probably do not represent the actual number of cells, I think they may be regarded as giving a fairly accurate comparison of the two sides. The total loss for the whole area was 50 per cent, and this was distributed to the three nerves as follows:

	PER	CENT
14th nerve	4	7.4
15th nerve	5	3.2
16th nerve	4	8.8

Many cells of successive sections, both in this and other specimens, were then measured with the ocular micrometer, and camera lucida drawings were also made and the dimensions taken, and while it was possible to demonstrate a difference in the size of the cells, the larger were found to be as often on the operated as on the unoperated side, and no abnormally small or otherwise defective cells could be demonstrated. Both methods of estimation, therefore, seem to indicate conclusively that the loss is in the number of cells.

The loss in the posterior horn is less, but still evident. Measurements showed that the distance from A to B (Fig. 14) is always less on the injured side and also that the distance from the center to the periphery in the dorsal part of the cord is slightly decreased. A quantitative estimate of this difference seemed impracticable.

Experiment 206. The period of incubation before operation was 3 days 1 hour. The wing, a small elevation as in experiment 538. The right wing was again removed, and the embryo preserved five days later, so that when examined it was 8 days 1 hour old. A slight thickening only was visible in the region of the

right wing, but aside from this defect the embryo was normal externally.

Sectioning showed that the scapula was entirely lacking, the clavicle nearly normal, and the coracoid and head of the humerus present but somewhat defective. On the uninjured side the same muscles were to be distinguished as in the last experiment, but on the operated side only the merest remnants are present, and these are so abnormal in shape and position, as well as in size, that it is difficult to identify them. Probably they are the pectoralis major, trapezius, latissimus dorsi, triceps, subclavius and deltoides. Fig. 4 is a photograph of a cross-section through the injured region.

The loss in the peripheral nervous system may be seen by comparing A and C of Fig. 33. The abnormality is much greater than in the last experiment, but this is to be expected because of the changed positions of the muscles as well as the increased loss in size.

Again it will be seen that wherever a muscle has been destroyed the nerve is also absent.

Measurements in this specimen show the following losses:

NERVE TRUNKS	•		THICKNESS PER CENT
14th		. 34.2	66.3
15th		. 21.4	20
16th		30	12†
VENTRAL ROOTS		WIDTH	THICKNESS
14th		. 20	334
15th		24	0
16th		. 20	11.1

	LATERAL	DORSO-VENTRAL	
	DIAMETER	DIAMETER	THICKNESS
GANGLIA	PER CENT	PER CENT	PER CENT
12th	. 21.3	16.7	12.1
13th	. 22.3	28.9	11.1
14th	. 16.7	74.9	35

The cells of the ventral horn were counted as before, showing losses as follows:

P	er crut
14th nerve	53 - 1
15th nerve	49.7
16th nerve	71.8

It will be observed that, while the percentage of loss in the size of the ganglia is not increased, is in fact slightly less than in the previous experiment, the loss in the ventral horn is greater, and this is exactly what would be expected, for the destruction of the muscles is much more complete, despite the fact that a slight projection of the wing remains, while the loss of sensory areas is not correspondingly great. The loss in the posterior horn is of the same nature as in experiment 538, and no attempt was made to determine this quantitatively. Fig. 15 is a photograph of a cross-section of the spinal cord in the region of the operation.

Experiment 225. This embryo was operated on after a period of incubation lasting 74 hours, and was returned to the incubator for 5 days 1 hour, making its age at the time of preservation 8 days 3 hours. A small portion of the wing was left, but the ventral portion of the body wall from the wing to the leg was not developed, and sections showed some distortion of the pelvic region. Detailed observations were, however, confined to the part anterior to the leg. Figs. 7 and 8 are photographs of this embryo.

The scapula was affected little if any, and a large part of the humerus was present; the muscles dorsal to these bones are normally developed. Only the most anterior portions of the coracoid and sternum are present and the muscles are as follows:—the subscapularis, deltoides, latissimus dorsi, triceps and pectoralis secundus are all present, but more or less abnormal; only remnants of the biceps and pectoralis major are to be found, while the teres, subclavius, pectoralis secundus and tertius are entirely wanting. Fig. 5 shows the chief bones and muscles.

The distribution of the peripheral nerves is shown in Fig. 34. Branches innervating the pectoralis and biceps are smaller on the operated side, and no trace of those normally reaching the missing muscles is found. Quantitative estimates of the loss throughout the injured region were not made for this specimen, but measurements were taken here and there and the results agreed with those obtained in the two previous cases studied. The defects in the spinal cord were also of the same nature, and are shown in Fig. 16.

The effect of the injury in the region between the wing and the

leg presents some new features. This area is developed from 6 somites, the 20th to the 25th inclusive, and each of the 6 nerve trunks innervating it has practically the same peripheral distribution. The course of these nerves is shown in Fig. 37, from which it will be seen that the nerve divides at once into a dorsal, a lateral, and a ventral branch. In this specimen the parts innervated by the dorsal and ventral branches are normal, but there are only remnants of the area to which the lateral branch is normally distributed, and in some cases it is entirely absent. From the conditions found in the brachial region it would be expected that the lateral nerve would be smaller or wanting, and this was found to be true. But the nervous system is further affected, for instead of 6 ganglia and nerve trunks, there are only 4. It is impossible to say which ganglia are missing, for this result is brought about partly by an increase in the thickness of those present, and partly by a greater distance intervening between successive ganglia. While the loss of the 2 ganglia is compensated for in some degree by the increased thickness, the area of the cross-sections of any ganglion is always less, and the nerves are decreased in size. As the beginning of both the ganglia and the peripheral nerves must have been established before the operation, it seems difficult to 'make it account for this condition. But as it occurred again in another ectopic embryo and only in this, it can hardly be regarded as a mere coincidence. Moreover, a younger embryo, to be described later, gives a clue to its meaning.

Another noticeable feature is the effect on the spinal cord (Fig. 17). There is no antero-lateral projection of the ventral horn in this region, and no definitely localized area of loss can be demonstrated, but the whole half is decreased in size while retaining its normal shape and proportions.

Examination of these three cases demonstrate clearly that as early as 5 days after the extirpation of peripheral areas well defined defects appear in the nervous system, but consideration of this period alone can give no conclusive evidence in regard to the process by which this condition was reached. Logically any one of three reasons might be assigned:

I The injury inflicted has caused this portion of the embryo to

develop more slowly, and the condition of the operated side is merely an earlier stage of development.

- 2 The missing nerve elements have formed, and then degenerated.
- 3 The neuroblasts are in some way dependent on the surrounding tissue, particularly their normal end organs, for development, and when these are wanting they do not differentiate.

The first hypothesis may be disposed of at once by comparing the defective cases with earlier stages of development (figured on the normal side of the experiments which follow), from which it will be seen that the condition in the operated embryos is not found at any time in the normal process of growth. Considering the stage of development that had been reached at the time of the operation, it is evident that if the second hypothesis is correct, the missing nerve elements must have both formed and degenerated within five days. And the degeneration must have been complete, for no cells in the process of destruction could be found. The rapidity of development in the chick makes this possible, perhaps, and the question could be decided only by an examination of earlier stages.

# Specimens Preserved from Four Days to One Day After the Operation

Three specimens were preserved on the fourth day after the operation. In one of these the injury was so slight that it was of practically no value. In another the spinal cord was apparently directly injured, and this was also discarded. In the third the operation and results were as follows:

Experiment 179. The egg was incubated for 66 hours, and at the time of the operation the wing bud was scarcely distinguishable. As a result of operating at this early stage it was found on examining the embryo 4 days I hour later that a small portion of the wing had not been removed, and that the body wall for a considerable distance posterior to the wing was entirely absent.

Only the merest remnant of the scapula was left and this was much out of position; the coracoid was present but abnormal in position, shape and size. The humerus was also abnormal, and probably the sternum, although this was not yet outlined with sufficient definiteness to make it certain. On the unoperated side all the muscles given in the previous experiments, though smaller and in some cases less definite, could be identified. On the operated side only the pectoralis major and the subclavius could be distinguished with certainty, but it is probable that two other muscles, small and out of position, are the trapezius and pectoralis secundus. For these points examine Fig. 6. Besides these, small masses of muscular tissue, entirely unidentifiable, are developed here and there, and mingled with them are masses of glandular tissue, which are apparently portions of the Wolfian body, for a connection can be traced to the main mass of this organ. Portions of other organs are also out of position, and the general confusion that results makes the tracing out of the peripheral nervous system on this side extremely difficult, for any muscular tissue, no matter how small the amount, nor how much out of position, attracts nerve fibers, but Fig. 35, A, represents the chief points. B shows the distribution on the normal side.

Measurements of the nerve trunks taken as described above show the following loss in width:

		r	ERCENT
14th nerve	`	 	22
15th nerve		 	30.3
16th nerve		 	28.5

The loss in the ganglia is shown by this table:

	LATERAL		DORSO-VENTRAL
	PER	CENT	PER CENT
Ganglion 12		20	14.8
Ganglion 13		39	32.9
Ganglion 14		12.7	14.1

The cells of the ventral horn were not counted, but after a careful comparison which showed that there was no appreciable difference in their size on the two sides of the spinal cord, the greatest width of the antero-lateral portion was taken, beginning at the end of the 11th ganglion and extending to the end of the 14th. A loss of 39.8 per cent in width was found. Through the greater part of this region, however, the distance from the central canal

to the inner border of the motor nucleus was greater on the operated than on the unoperated side. This is shown in Fig. 18, a photograph of a section of the spinal cord at the level of the 15th nerve. An explanation of this may perhaps be found by assuming that the normal number of neuroblasts were formed, but part of these were arrested in their development, and never reached their normal location in the ventral horn. As before, while the loss in the dorsal horn is evident, it is impossible to arrive at any satisfactory quantitative estimation of it.

As mentioned above this embryo was also ectopic, and showed the same peculiarity as Experiment 225 in the loss of ganglia and nerve roots between the wing and the leg. Unfortunately the posterior portion of this specimen was destroyed, and the number lost is therefore uncertain, but as far as the series goes there are 7 post-brachial ganglia on the normal side and 5 on the abnormal. As in Experiment 225 there is an increase both in the thickness of the ganglia, and in the number of intervening sections, but the dorso-ventral and lateral diameters were taken throughout the course of ganglion 16, and from these the average loss in area for each section was estimated to be about 25 per cent. Measurements of the succeeding ganglia were taken at intervals, and showed a constant loss; percentages were not The peripheral distribution of these nerves on the normal side agrees with that shown for Experiment 225, while on the injured side the lateral branch is missing or defective according as the lateral musculature is missing or defective.

Although the spinal cord changes its shape in different parts of its course between the wing and the leg, the condition represented by Fig. 19 may be taken as typical of the character of the loss. This is more difficult to localize than in the brachial region, for the motor nucleus is not so clearly defined, but examination with a high-power lens shows that less neuroblasts have differentiated into the characteristic motor cells. The dorsal horn is also smaller, and all the dimensions of the spinal cord decreased.

Experiment 324. This embryo was preserved 3 days 6 hours after the operation and was then 6 days 19 hours old. The amnion was grown to the embryo in the region of the wound, and in

attempting to detach it the surrounding tissues were somewhat torn. Still it was possible to identify the muscles and trace the course of the nerves.

Not all the muscles are yet clearly defined, but of those which are, the pectoralis secundus, the subscapularis and latissimus dorsi were entirely extirpated by the operation. The humerus and coracoid are abnormal and the scapula completely destroyed. The peripheral distribution of the nerves is shown in Fig. 36. Measurements of the nerve trunks show an average loss of 31½ per cent in width. The ganglia are not noticeably affected. The dorso-ventral diameter of the spinal cord and the lateral diameter of the ventral portion are less on the operated side, but the posterior horn exhibits no change. The boundaries of the ventral horn on the operated side are, in many sections, less sharply outlined, and exact measurements are difficult to obtain, but a very conservative estimate of the average loss in width is 18 per cent. As in Experiment 179 the distance from the neural canal to the motor nucleus is sometimes greater on the operated side, and as this is the case where the boundaries are least definite, I again assume it to be possible that certain neuroblasts were formed which did not reach their normal location in the ventral horn. A section of the spinal cord at the level of the 15th nerve is shown in Fig. 20.

Experiment 404. Of specimens preserved when five days old, or two days after the removal of the wing bud, I shall again confine myself to a detailed description of a single embryo, since others of the same age differ only in the extent of the injury. At this period the bones and muscles are not yet sufficiently differentiated to be identified, so that it is impossible to say exactly what structures have been destroyed, but there is only the slightest prominence in the region of the wing on the operated side. Fig. 9 will show the stage of development as well as the extent of the injury.

Fig. 38 shows the peripheral distribution of the nerve fibers. The branching on the operated side is decidedly less extensive, yet I am unable to demonstrate more than 4 per cent of loss in the average width of the nerve trunks, and I do not feel certain that this is not within the limits of error in measuring these with the

ocular micrometer. The effect on the ganglia is more evident, and the loss is distributed as follows:

DO	RSO-VENTRAL	LATERAL
	DIAMETER	DIAMETER
	PER CENT	PER CENT
Ganglion 12	I	9
Ganglion 13	. 16.4	11
Ganghon 14	3+	3+

The areas within the spinal cord are not sufficiently differentiated to make measurements practical, but Fig. 22, a photograph of the spinal cord in the region of ganglion 13, shows a loss in the ventral horn. The posterior horn seems to be entirely unaffected.

Fig. 23 is a cross-section of the spinal cord in Experiment 79, which was preserved, at the same age as Experiment 404, and agreed with it in all essentials. Other specimens at this stage of development showed the same conditions.

In Experiment 142, killed 24 hours after the operation, in which when examined there was no external evidence of the wing except a slight thickening of the body wall, I am able to demonstrate but a very slight difference in the development of the nervous system on the two sides, and this is found in a decrease in the extent of the peripheral branching and a very slight decrease in the size of the ventral horn. At this period the beginning of the differentiation of bones and muscles is evidenced by aggregations of cells into more dense masses, but no individual structures can be identified. The nerve trunks have reached the inner margin of the wing, and are beginning to branch within it. These conditions are illustrated by Figs. 13, 39 and 24.

Other embryos killed at this period always show a decrease in the extent of the branching of the nerves, but in some cases there is no appreciable effect on the spinal cord.

# E. Experiments at Later Stages of Development

The next experiments were designed to test the effect of removing the wing at different stages of its development. I am indebted to Professor Lillie for materials for Experiments 5 and 10, the

operations having been performed by him in the investigation of another problem.

#### 1. After Six Days of Incubation

Experiment 10. This embryo was 6 days old when the wing bud was amputated, and when preserved 4 days later it was found that only the distal part of the limb had been removed, a stump extending nearly to the elbow being left. Although there were some abnormalities in position, shape and size of other parts, it will be seen by consulting Fig. 33, A, that the principal nerves involved would be the branches lettered fw and fw. As the loss would necessarily be small in amount this embryo was not studied with much detail, but there was a slight but evident defect in the ventral horn in the region of the 14th nerve.

# 2. After Five Days of Incubation

Experiment 5. The period of incubation before the operation in this case was 5 days, and when examined 2 days later it was found that a small stump was left in the region of the right wing. By referring to the figures for the normal side of Experiment 404, which was approximately in the same stage of development as this embryo at the time of the operation, it will be seen that the branches of the peripheral nerves must have been sectioned by the operation. The chief point to be gained from this embryo is that there is evident degeneration of the fibers of the pectoralis, biceps, and triceps nerves. The degeneration extends for only a short distance toward the center and no abnormal cells are found in the motor nucleus.

This is, however, smaller; so that there was evidently a lack of development as well, and this would be expected, for the motor nucleus does not appear to become complete and separated as a distinct unit from the rest of the spinal cord before the 6th day. Cajal states that it is complete by the 5th day, but as the stage of development which he has figured for the 5th day differs but little from that which I have regarded as typical for the 6, the discrepancy probably arises from the difficulty in timing the actual period after fertilization. Moreover the defect in the motor nu-

cleus is less than it is in other specimens where the peripheral areas destroyed were no greater, so that both degeneration and lack of development must doubtless be taken into account.

### 3. After Four Days of Incubation

Experiment 19. This egg was incubated 4 days before the wing was removed, and preserved one day later. Only a slight elevation was left in the region of the right wing, otherwise the embryo was normal. There was no demonstrable effect on the nervous system except that the branching of the peripheral nerves was less extensive and in some sections the ventral horn was probably slightly decreased, a condition similar to that found when 3-day-old embryos were operated on. There was no degeneration of fibers or cells, although the ends of the nerve trunks were probably sectioned by the operation. This condition I shall attempt to explain later.

#### F. Removal of Somites

I then decided to study the effects of operating at an earlier stage by removing somites. By choosing a period when the primordium for all the muscles of a given somite is still to be found in the muscle segment, and destroying this, all the motor organs normally innervated by a given nerve would be extirpated. (The only possible exception being areas reached by the sympathetic system.) As nearly as could be determined, this condition is found in the wing region of the chick from about the 45th to the 60th hour of incubation. At this period there is no visible differentiation within the medullary tube, and no outgrowth of peripheral fibers. It would therefore be possible to study the differentiation of the motor nucleus and motor root from the very beginning of their formation in the entire absence of all muscles which they normally innervate.

There are many technical difficulties to be overcome in experiments of this kind, but with suitable apparatus, and some experience, it was found possible to remove the primordium of all the musculature of a given somite without injury to the spinal cord. A Zeiss binocular microscope was used in order to magnify the somites so that they could be counted, and their extent determined without inverting the object. In order to get sufficient light for this within the egg-shell it is necessary to have either very bright sunlight or an arc lamp with a condenser. The medullary tube and neural crest are very close to the myotomes at this period and the dorsal aortæ are nearly beneath, so that it requires the utmost care not to injure the one or the other. A mechanical method of removing the somite intact would be desirable, but I was unable to devise any means of doing this without injuring the adjacent parts, and I again resorted to electricity. To insure the exact location of the needle an apparatus for holding the electric handles, devised at the University of Chicago, and described by Patterson in the Journal of Morphology for April, 1909, was used.

Experiment 60. This egg had been incubated 53 hours before the operation, in which I attempted to destroy the three brachial somites, and was preserved 50 hours after it. As my arrangements for lighting were at that time very inadequate the extent of the injury had to be judged by its effect: About the same areas were destroyed as when the wing bud itself is removed at its first appearance (Fig. 10) and the effect on the nervous system is practically the same. The peripheral branching is less extensive on the injured side (compare A and B in Fig. 41) and the ventral horn is slightly smaller, both of which conditions obtain in embryos operated on at 3 days and preserved 2 days later.

Experiment 84. In this embryo the operation was performed after 45 hours of incubation, and it is evident that all the musculature of the second brachial somite was destroyed. It was preserved 24 hours later. There is a slight injury to the most dorsal portion of the spinal cord, but as the evidence that the direct injury was confined to this region is so unmistakable, I have not hesitated to regard this specimen as furnishing conclusive evidence concerning the behavior of motor neuroblasts in the absence of all muscles which they normally innervate. Moreover the ganglia are wanting on both sides throughout the brachial region, and as, at the time of operation, the ganglionic crest would lie in close contact with the dorsal portion of the spinal cord, it was doubtless

directly destroyed. This embryo, therefore, offered a favorable opportunity to study the behavior of the motor nerves alone.

On the normal side the nerve trunk, though smaller than when the sensory root is also present, has reached the inner margin of the wing, the typical condition at this stage of development. (Fig. 2.) The fibers on this side follow a straight course and form a compact mass as usual. On the operated side, the ventral horn is much smaller but some fibers are formed; these, instead of following a straight path through the musculature to the region where the inner margin of the wing would normally be found, end in an irregular mass a short distance from the medullary tube. (The same condition was found in Experiment 69 and is represented in Fig. 40.)

Experiment 69. In this specimen, preserved 24 hours after an attempt to remove the three brachial somites, there were two elevations in the region of the wing with a depression between them, but these were together much smaller than the normal wing. On sectioning I found that the myotome of the first brachial somite was entirely missing, but there was some lateral outgrowth of tissue to form the wing. In the second somite there was no myotome, and practically no thickening of the wall in the wing region. In the last somite both the myotome and the wing bud were nearly normal. There had again been a very slight injury to the spinal cord in the most dorsal portion and as the brachial ganglia were entirely wanting it is probable that the neural crest was destroyed by the operation.

The conditions in the second brachial somite were practically identical with those described in the last experiment with the possible exception that a slight thickening of the body wall may contain a very small amount of muscle tissue, not yet sufficiently differentiated to be identified. But as there is no evidence of the myotome, which should be prominent at this period, and the nerve fibers behave exactly as in Experiment 84, it seems probable that absolutely all of the primordium of the muscle cells was destroyed. The ventral portion of the spinal cord and the path of the nerves on the operated side are represented in Fig. 40. The nerve trunk on the operated side follows the normal course in direction and extent, but again lacks the sensory fibers.

From these two experiments, and others which are not so conclusive, but which furnish evidence corroborating these results, I think it is safe to conclude that even when the primordium of all the musculature of a single somite is destroyed, some motor cells and motor fibers will develop within the medullary tube of this segment. They will be fewer in number than on the normal side, they will extend a shorter distance from the spinal cord, and instead of forming a nerve trunk with well-defined boundaries, in the initial stages, they will run freely into the mesenchyme, forming an irregular mass. Their behavior in later stages of development is shown by the experiment which follows.

Experiment 706. The period of incubation was 50 hours, and an attempt was again made to destroy the 17th, 18th and 19th The embryo was examined and preserved 92 hours later. Figs. 11 and 12 give the external appearance. There is a slight elevation in the anterior part of the wing region on the operated side, and examination of sections shows that a portion of the 17th myotome is left, the 18th is entirely wanting, and only a fragment of the 10th is present. No brachial plexus was formed on the operated side, and each nerve must therefore be treated separately. The 12th ganglion is smaller than on the normal side, as is also the corresponding ventral horn, and the course of this nerve, the first brachial, is shown in Fig. 42, A. There is no distinct ganglion for the somite following this, but in the place where it should appear there is a clear space much the shape of a ganglion (Fig. 21), containing occasional cells which appear to be of a ganglionic character. These become more numerous posteriorly and finally blend with the 14th ganglion, which is abnormal in shape. The interpretation of this would seem to be that the cells of the 13th ganglion, which would probably have been partially formed at the time of the operation, have been attracted to the region of the 14th, and have united with it. There is no distinct nerve root in this somite, but there is a motor nucleus and most of the fibers arising from these cells pass out at various levels of the spinal cord to join the third brachial nerve. A few fibers end freely in the mesenchyme, as in Experiment 69, a short distance from the spinal cord. The ventral horn of the third somite is smaller on

the operated side, as is also the ganglion, but the nerve is not noticeably smaller, due presumably to the addition of fibers from the somite above. Most of this nerve passes down to the next somite as the sympathetic branch, which is much larger on this side than it is on the normal side. Its course is shown in Fig. 42, B. The effect on the spinal cord is illustrated by Fig. 21, a cross-section in the region of the 15th nerve.

In this process of the union of two ganglia and nerve roots is to be found an explanation of the condition described in Experiments 225 and 179, where, in the injured region, there were but four ganglia and nerves instead of six, while no one region of the spinal cord was more defective than another. As was shown in experiments 69 and 84, some nerve fibers do develop in every somite even when all the end organs of the motor cells are destroyed and the sensory area much decreased. (It is impossible to have a region in which there may not be some sensory tissue.) These fibers at first wander freely in the mesenchyme without forming a compact nerve, but are later presumably attracted in the direction of the end organs above or below, and finally the ganglia and nerve roots unite. This process had been completed in Experiments 225 and 179, without leaving any direct evidence of its having occurred.

### G. Summary

- I The complete destruction of the primordium of any muscle before its innervation results in the complete suppression of the branch of the peripheral nerve leading to it.
- 2 Complete destruction of all the muscles of a given somite does not, however, lead to the complete suppression of all the motor cells and motor fibers at this level of the spinal cord, but the number is very greatly reduced, and these present innervate muscles of the somite above or below.
- 3 The nerve leading to a defective muscle resulting from the destruction of a portion of its primordium before innervation is always decreased in size, but it is usually larger in proportion to the size of the muscle than the normal nerve is in proportion to the size of the normal muscle. This holds true even if the most minute fragment of the muscle is left, and in general it may be

said that the smaller the muscle, the greater is the proportionate size of the nerve.

- 4 Muscle fibers in abnormal positions without any apparent attachment to the skeleton, always receive innervation.
- 5 Except in the early stages of development no nerve fibers are ever found wandering free in the mesenchyme, but they follow a definite path leading to a muscle or other end organ.
- 6 Decrease in the number of the peripheral motor nerve fibers is always accompanied by a decrease in the size of the ventral root. In some cases, an extensive destruction of musculature leads to the union of two ventral roots, and a consequent decrease in the number of nerve trunks.
- 7 Defects in the size of the motor nerves are always accompanied by a corresponding defect in the ventral horn of the spinal cord, but in no case is the latter entirely lacking, even when the musculature of the somite in which it is located has been completely extirpated.
- 8 In some specimens the distance from the central canal of the spinal cord to the inner edge of the ventral horn is greater on the operated than on the unoperated side, and the line of demarcation between the ventral horn and the surrounding cells is less definite.
- 9 Extirpation of sensory areas has the same effect on the spinal ganglia and dorsal roots that the extirpation of motor organs has on the ventral roots and motor nucleus. In case the ventral roots are united the ganglia and sensory roots are also united.
- 10 A decrease in the size of the ganglia is accompanied by a decrease in the size of the dorsal horn, but this effect is not observable until a somewhat later period.
- II In regions where the ganglia and nerve roots of two successive somites unite, the entire half of the spinal cord is slightly decreased in size, but there is no definitely localized area of loss.
- 12 Effect on the spinal cord is mostly confined to the region from which the nerves innervating the extirpated parts arise, but slight defects both anterior and posterior to this level are usually observable.
  - 13 The first defect in the nervous system is demonstrable about

24 hours after the operation, its extent and character depending on the extent of injury to the periphery. If only the brachial musculature is removed no effect manifests itself at this time except a slight decrease in the extent of the peripheral branching, and a more doubtful decrease in the size of the ventral horn. If all the musculature is destroyed no definite nerve trunks are formed, but the fibers wander free in the mesenchyme and the ventral horn is more defective.

- 14 The effect of operations performed on the second or third day differs only in the amount of musculature involved.
- 15 In operations at a later period, when connection with the end organs has already been made, and therefore involve sectioning a nerve already formed, degeneration may occur. This starts at the peripheral end of the nerve and extends slowly toward the center, so that the spinal cord is affected at a much later stage than it is when the operation causes inhibition of growth.
- 16 In operations performed on the fourth day, since no degeneration occurs when the destruction of the musculature is not complete, the nerves already formed and sectioned by the operation would seem to be directed to the peripheral organs which are not destroyed.
- 17 No degenerating fibers can be observed when the extirpated areas are removed before the nerves reach the region of the operation, and in no specimen studied has degeneration reached the nerve cells; so that it may be added as the final demonstrable fact that—
- 18 Under the conditions of the experiment, the defects which appear in the nervous system are not due to degeneration, but to the failure of the neuroblasts to develop.

#### H. Discussion

In discussing the bearing of these facts on the problem of the differentiation of the neuroblasts, I shall limit myself, for the sake of clearness, to a consideration of motor neurones, for the extent of an injury to the musculature can be more sharply defined than an injury to sensory areas, and the process of differentiation of the motor neuroblasts can be more easily followed.

So far as the process of differentiation of a motor neurone can be observed, it consists in the movement of the neuroblast into the region of the ventral horn, increase in its size and change in the shape of the cell itself, and the development of a fiber. motor cell which is entirely self-differentiating would, under the conditions of the experiment, reach the ventral horn and develop a fiber, for this region is uninjured and the food supply unaffected. But as in some cases the motor nucleus and motor roots are not more than 25 per cent of their normal size, and as it has been shown that this is not due to degeneration but to failure to develop, it is clear that at least 75 per cent of the motor cells are influenced in their development by the presence or absence of the muscles which they normally innervate. Moreover, as 3 or 4 days after the operation there are more undifferentiated neuroblasts on the injured than on the uninjured side, and the cells which are found in the ventral horn are perfectly normal, it seems equally apparent that even the initial stages of differentiation are wanting, and that these cells are entirely dependent on the presence of their muscular end organs for differentiation.

But in every specimen examined, even when there is no trace of musculature in the somite concerned, some motor cells do differentiate completely. We are then forced to sne of two conclusions:—either we have two classes of motor newroblasts, one of which is entirely dependent on stimulation from the periphery for differentiation, and the other entirely independent of such stimulation; or the neuroblasts which do develop normally are influenced by the musculature of adjacent somites. It seems improbable on purely a priori grounds that such a wide difference in the physiological activity of cells having a similar origin, and normally a similar destiny, as the first assumption postulates, can exist. if it is to be assumed that the muscles of other somites are a source of stimulation, it is perhaps necessary to show that the nature of the stimulus may be such that it would naturally be expected to extend to other regions of the spinal cord than that from which the nerves innervating these muscles normally arise. This I shall attempt to do by considering some of the factors involved in differentiation.

The essential feature in the differentiation of any cell must be a change in its protoplasmic structure, and the possible factors involved in this change may be reduced to three, the protoplasm of the cell itself, the food supply, and chemical substances or physical forces coming either directly or indirectly from the medium immediately surrounding the cell. It is obvious that under the conditions of the experiment the food supply is not affected and is, therefore, the same for both the neuroblasts which differentiate and for those that do not, and that the change in the protoplasmic structure of the cell must be due to the way in which the food is assimilated. The manner in which assimilation takes place is still hypothetical, but the most probable theory yet advanced is that the blood proteids are broken down by enzymes or other chemical substances and the active amino acids or other radicals then recombine after the pattern of the cells of the tissue involved. That they recombine differently in different cells is due to the fact that the blood proteids are broken down at different points, and this is in turn due to the presence of different kinds or amounts of enzymes or other activating chemical substances. When differentiation occurs, unless a vital force is postulated, it is necessary to assume that the kind or amount of enzymes must have been increased or decreased, or some other chemical substance or substances have been added or subtracted. The cell during its whole embryonic history has been repeating the same cycle of processes, namely, assimilating food in a definite way, increasing in size and dividing, and it is impossible to conceive that any tendency to develop in a certain direction, any adaptation to conditions, or any need of the organism can produce a new chemical substance or inhibit the action of one already present. Differentiation of any cell must therefore occur because of a change in the chemical composition or physical properties of the lymph surrounding it.

In case of the neuroblasts, the cells outside the medullary tube are also differentiating and the products of their metabolism must change, either in kind or amount, and these products must enter the lymph. It is therefore evident that the presence or absence of muscles in a given somite must influence the character of the

medium surrounding the neuroblasts in its immediate neighborhood, and thus a change in the chemical inter-reactions may be effected. This would give at least a possible explanation of the influence of the muscles on the nerves by which they are normally Moreover, although the metabolic products of muscular activity would necessarily be found in greatest amount in the somite in which the muscles were located, with the free exchange of lymph they must also affect, to some extent, the character of the lymph in adjacent somites. This would give a perfectly natural explanation for the differentiation of some motor nerves in somites in which the primordium of all the muscles had been extirpated. And it is very significant that in such cases, when the fibers are sufficiently developed for connection with the end organs to be apparent, they are found to innervate structures in the somite above or below. I therefore regard the development of some motor nerves in regions where the muscles which they normally innervate have been destroyed, as in no way inconsistent with the belief that neuroblasts do not differentiate into motor nerves without the presence of muscular end organs, but rather as a necessary corollary of this condition. And as these experiments clearly demonstrate that the majority of neuroblasts which normally develop into motor nerves show no sign of differentiation in the absence of their end organs, I conclude that in the chick all such neuroblasts are entirely lacking in the power of self-differentiation.

#### III EXPERIMENTS ON AMPHIBIANS

# A Historical and Critical Survey of Previous Work

It is a universally accepted principle in biology that the fundamental properties of protoplasm are essentially the same for all organisms, otherwise it is useless to attempt to formulate any general laws governing the animal world. And whether an organ or a cell attains its adult condition because of powers inherent within itself, or whether it must be acted on by outside forces to reach this condition, must be regarded as fundamental. Yet other investigators, working on different forms, have reached the conclusion that the neuroblasts are entirely self-differentiating, and one

is naturally led to inquire if there is any possibility of placing a different interpretation on these observations.

Harrison's experiments, described above, in which portions of the medullary tube placed in a drop of lymph developed nerve fibers, are open to the objection that the lymph necessarily contained products of the metabolism of various organs of the body, and it is therefore not certain, indeed it is improbable, that the neuroblasts were removed from the influence of end organs whose physiological activities were similar to those which they normally innervated. Such an experiment is, therefore, not crucial, though I believe it furnishes the strongest evidence in favor of self-differentiation that has yet been given. The experiments in which pieces of the medultary tube were transplanted to abnormal positions inside the body of the tadpole are open to the same objection to a still greater degree.

A number of points require further investigation before Braus's experiments in removing the limb of Bombinator can be regarded as proving conclusively that the neuroblasts are self-differentiating. His conclusions rest mainly on the fact that ten days after the removal of the limb the brachial plexus is as large on the operated as on the unoperated side of the animal, although some weeks later, after metamorphosis, it is smaller. "Die Verschmächtigung bei dem älteren Objekt," he says, "ist demnach eine sekundäre." But before this can be accepted the degree of differentiation of the nervous system ten days after the operation must be considered, and the impossibility of stimulus from any brachial muscle or other muscles be established.

In regard to the specimens examined near the end of metamorphosis he says, "Derselbe (the brachial plexus) ist allerdings dünner als derjenige der normalen Seite der Larve und auch ventralwarts mit Muskelanlagen der Bauchwand in Verbindung." In the specimen killed 10 days after the removal of the limb he states that the trapezius and interscapularis muscles are present, so that in neither of these cases are the neuroblasts removed from all muscular influence, and experiments on the chick have shown that the defect in the size of the nerves is not proportional to the defect in the musculature. A certain degree of development may

thus be accounted for, and it cannot be asserted that the condition found in the older embryos is due to degeneration rather than to lack of development, unless there is evidence of degeneration, or it can be shown that the differentiation of the motor horn is once completed, and the motor roots at some period contain the full number of fibers, for the question of self-differentiation must be reduced to a consideration of individual cells, and under the conditions of Braus's experiments any neuroblast which normally differentiates into a motor nerve, and failed to do so here, could not be self-differentiating.

# B Purpose, Methods, and Material

The following experiments were conducted for the purpose of making further observations on this point:

Obviously the method of studying this problem by extirpating limb buds cannot give crucial results with any animal in which the regeneration of these parts occur, and I have not succeeded in getting amphibian material in which the limbs are not regenerated repeatedly. In my experiments I found that Bufo americanus regenerated less rapidly, and perhaps less completely, than Amblystoma tigrinum or Rana pipiens, and probably in no instance were all vestiges of musculature connected with the limb removed. Whatever the cause, I have no specimen which was allowed to live long enough for regeneration to be expected in which it did not occur. Nevertheless I believe that the results obtained indicate that the process of differentiation of the neuroblasts is essentially the same in the amphibians as it is in the chick.

Amblystoma tigrinum, Rana pipiens, and Buso americanus were used. With Amblystoma, sometimes the fore limb, sometimes the hind limb, and sometimes both were removed, either when the limb bud was just apparent or before it had attained any considerable size, and in many instances the operation was repeated as often as the beginning of the formation of a new limb bud could be detected. The limbs develop slowly, and in the early stages a large proportion of the musculature innervated by the sciatic plexus forms such an intrinsic part of the body of the tadpole that the removal of the projecting portion of the limb leaves many muscles

quite intact. This fact, taken in connection with the constant and apparently immediate regeneration, makes it necessary to remove the limb a number of times before there is any considerable difference in the amount of musculature on the two sides. But the differentiation of the nervous system is equally slow and it is possible to materially decrease the muscular mass before the motor nerves are well developed. The toad was treated in the same way, but regeneration occurs more slowly, and differentiation of the nervous system more rapidly, so that I have no specimen in which the limb was removed more than twice. With the frog, I shall mention only experiments in the removal of somites.

The methods used to ascertain the extent of the defect in the nervous system were the same as those employed in studying the chick embryo.

# C Experiments on Amblystoma

Experiment 25. The left hind-leg bud was first removed May 3 and again successively on May 22 and June 1. The larva was preserved June 5. Some idea of the difference in the two limbs at this time may be obtained from Fig. 25. The bones of the pelvis are present but smaller on the operated side, and a small portion of the femur is distinguishable, but there is no evidence of any of the other bones of the limb. For the purpose of the present problem it will be convenient to divide the muscles innervated by the sciatic plexus into three groups: (1) The proximal muscles of the thigh; (2) the muscles of the leg proper; (3) the muscles of the bladder and its immediate neighborhood. In this experiment, groups (1) and (3) are uninjured. Of group (2), there is a considerable muscular mass, but it is impossible to identify individual muscles, so that it can only be said that the amount of musculature is much decreased. The sensory area is, of course, also decreased.

The sciatic plexus is formed from the union of three nerves as shown in Fig. 43, A, drawn from the normal side of this larva. Nerve I innervates mainly the proximal portion of th. thigh, and as this region is uninjured, only enough of its course is given to indicate the branch which joins the sciatic plexus. B drawn on the

same scale as A gives the course of these nerves on the injured side. The sciatic plexus itself, at the point marked (4), is about 75 per cent smaller on the operated side, and branches (1) and (2) are proportionately decreased. The branch marked (3) goes to the bladder and is of normal size. Following backward to the individual nerve trunks which form the plexus, II is found to be 40 per cent and III, 64 per cent smaller on the operated side. Of the two ganglia which correspond to these nerves, a rough but conservative estimate shows one to have lost at least 50 per cent in volume, while the other has lost about  $33\frac{1}{3}$  per cent. Fig. 27 was taken from a transverse section of the spinal cord in the region of the second nerve of the sciatic plexus. This is fairly typical of the cord throughout the injured region, although occasionally a section is found in which there is little difference between the two sides.

But while there is a decided defect in the nervous system at this period, 33 days after the first removal of the limb, from this specimen alone it is impossible to determine whether it is due to degeneration or to failure to develop, although there is no evidence of degeneration. As will be seen from the figures the nerve trunks are, at this time, well established, and differentiation within the spinal cord, if not complete, is at least well advanced. It is therefore necessary to examine younger specimens for signs of degeneration, and to determine as nearly as possible the period at which differentiation of the motor neurones is complete.

Younger Embryos. I found that the length of time which must elapse after the operation before a defect in the nervous system appears depends on the degree of differentiation attained, and neither age nor size is an exact criterion of this. Size is a much better measure, but my records were made with time as the standard, with some comparative data in regard to length and the rapidity of growth. In one specimen in which the limb was slightly larger than usual at the time of its removal, and in which the larva continued to grow rapidly, a slight diminution in the size of the motor horn can be demonstrated at the end of ten days. Fig. 26 is typical of the spinal cord in the operated region of this embryo, but there are sections in which no difference in the two

sides can be detected, and even a few in which the motor nucleus is larger on the injured side. On the whole, however, there is no doubt but that the ventral horn of the normal side is larger. The brachial plexus is also somewhat decreased in size. Aside from this specimen, I have no other in which the nervous system is noticeably affected until 14 days after the removal of the limb. The following experiment will serve to illustrate this condition:

Experiment 27. The forelimb, in which the beginning of the formation of two toes could just be detected, was removed May 3, and the specimen was preserved May 17. The animal grew slowly and regeneration was correspondingly slow, but evident. At the time of preservation the nerves have entered the limbs, but are still little developed, the main trunk being but .03 mm. in diameter in the widest part. The motor roots are very minute, and the motor horn is just beginning to be perceptible as a distinct unit. Figs. 28 and 29 are taken from a cross-section in this region. The defect shown in the spinal cord is rather extreme, the difference between the two sides not being quite so great in most cases.

I have no specimen younger than this, except the one just cited, in which the nervous system is not developing normally, and I have older specimens in which it is also normal. But in all of these cases some of the limb muscles are present, and regeneration is going on, so that the amount of loss in the mass of the musculature is comparatively small, and the point I wish to establish is that when the peripheral area is extensively injured a defect appears in the nervous system before differentiation is completed. Comparing the nervous system of the normal side of these larvæ which were killed respectively, 10, 14, and 33 days after the removal of the limb, it is evident that there are less fibers in the nerve trunks and nerve roots, and less cells in the motor horn of the two former. Since a defect has already appeared, there can be no time at which all the neuroblasts which normally differentiate into motor nerves are found together in the motor horn.

Have the missing motor cells been formed and then degenerated? To answer this I shall compare the larva in Experiment 27 with another of the same age, kept under the same conditions, and apparently developing parallel with it, but which was killed nine

days after the removal of the limb. In this specimen the normal nerve roots can be traced but a short distance beyond the spinal cord (Fig. 30), and I cannot demonstrate with certainty the presence of any motor fibers, but there is some evidence of the beginning of the formation of the ventral horn. Only five days elapsed between the time when this larva was killed and the one in Experiment 27. There is no sign of degeneration in the latter case, and when it is remembered that, so far as is known, the atrophy of tissue requires a longer period of time than its formation (compare surgical literature and experiments described by Bethe and others, and Experiment 5 on the chick embryo, for this point) there seems little possibility that, at the rate of development of this animal, these cells could have been formed and completely degenerated. Moreover, as the extent of the defect is continually increasing, if all the motor nerves are self-differentiating, there must be a continual degeneration, and tissue in the process of destruction is so apparent that it could not have escaped detection in some of the specimens studied. I can therefore see no reason for believing that the defects in the nervous system of the specimens studied is due to degeneration.

# D Experiments on Bufo lentiginosus Americanus

Little need be said in regard to the experiments on the toad, for they furnish no new points of interest. The presence of some muscles, and the fact that regeneration occurs, makes it impossible to study the behavior of the nerves in the absence of all the organs which they normally innervate. But in one specimen preserved nine days after the removal of a hind limb, there was a defect in the motor horn, although it was not yet completely formed on the normal side. Examination of several specimens showed that the motor horn and ventral root are always smaller if there is a marked loss in the mass of musculature, and no evidence of degeneration could be found. The toad, therefore, confirms the results obtained by experiments on amblystoma.

# E Removal of Somites in Rana Pipiens

I thought it possible that the capacity of the frog for regeneration might not extend to the reproduction of a whole somite, and I therefore tried removing these as a means of getting at the initial stages in the development of the nerve fibers. For this purpose larvæ were removed from the egg capsules from 24 to 36 hours before hatching, and from 1 to 3 somites completely removed. It is perfectly easy to do this without injuring other parts, but the animals all died three or four days later; however, by leaving a small amount of tissue close to the spinal cold and notochord they develop as rapidly as the normal larvæ, and after a very few days there is no noticeable external defect.

On sectioning these the loss is evident, but regeneration and probably re-differentiation of adjacent parts is found to occur, for no muscles are missing, but all are decreased in size and this loss extends to somites above and below the injured region. process is well advanced before there is any well-defined differentiation of the medullary tube, so that again it is impossible to ascertain the effect of the complete lack of the end organs of any nerve. Fig. 31 shows the condition of the musculature in a specimen preserved two months after the removal of the somites, and Fig. 32 shows the spinal cord of this section more highly magnified. The defect in the ventral portion of the spinal cord is evident throughout the affected region, and comparison with older larvæ shows that neither the motor horn nor the ventral roots have attained complete development. These were also examined for signs of degeneration, but none were found, so that these results again confirmed the conclusions reached by experiments on Amblystoma.

# F Summary of Conditions Observed in Amphibians

- I If a limb bud of Amblystoma tigrinum or Buso americanus is removed the following conditions are found:
- a If the regeneration is rapid and extensive the development of the nervous system is apparently normal.
  - b If the regeneration is slight, or if the limb is repeatedly

removed, so that there is a considerable loss in the amount of musculature, the nerve trunks, ganglia, and that portion of the spinal cord in direct communication with the injured area are defective.

- c The extent of the defect depends both on the amount of musculature destroyed and the degree of differentiation of the spinal cord at the time when the larva is preserved. In some instances a number of branches of the nerve trunk are entirely wanting.
- d There is no appreciable effect on the medullary tube until differentiation is apparent, but the effect always appears before the development of the ventral horn and ventral roots is complete.
- e No nerve fibers are found wandering free in the mesenchyme, but they always follow a well-defined path leading to a muscle or other end organ.
- 2 If the greater portion of a somite or somites is removed from a larva of Rana pipiens shortly before hatching, regeneration occurs but the amount of musculature is defective, and the nervous system is affected in the same way as that of the amblystoma or the frog when a limb bud is removed.
- 3 In any of these cases it is impossible to demonstrate degenerating nerve cells or nerve fibers, and the defects must therefore be due to the failures of these to develop.

## G Conclusions

Comparing the data obtained from experiments on amphibians with that obtained from the study of the chick embryo, it is evident that they agree in all essentials; in both, the destruction of a peripheral area leads to a defect in the nervous system, which is due, not to degeneration, but to the failure of certain neuroblasts to differentiate normally. The difference in the extent of the defect and the length of time required for its appearance are obviously due to regeneration, and to the slow rate of differentiation of the nervous system in amphibians. And we are again forced to conclude, either that there are two classes of neuroblasts, one of which is self-differentiating and the other not, or that these which do develop normally are stimulated to do so by the presence of end

organs. In every specimen that I have examined it is certainly possible that the neuroblasts which develop into motor nerves may have been influenced by the regenerating muscles which they normally innervate, or by other muscles in the immediate neighborhood, and in no case cited in the literature which has come under my observation has this possibility been excluded. I believe, therefore, that further evidence is necessary before it can be asserted that any neuroblasts are self-differentiating, and until such evidence is produced, that it is safe to assume that all are alike dependent on stimulation from end organs or the products of the activities of end organs, for differentiation.

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#### PLATE I

- Fig. 1 External view of normal chick embryo after 74 hours of incubation. w, wing bud.
- Fig. 2 Cross-section through the brachial region of the embryo shown in Fig. 1. g, ganglion; m myotome; n, end of nerve trunk; w, wing bud.
  - Fig. 3 Cross-section through the brachial region of the embryo in Experiment 538.

bi biceps

ps pectoralis secundus

cb r coraco-brachialis

pt pectoralis tertius

dt deltoides

ss supraspinatus

ld latissimus dorsi

sup subscapularis

pm<sub>j</sub> pectoralis major

ti teres et infraspinatus.

- Fig. 4 Cross-section through the brachial region of the embryo in Experiment 206.
- Fig. 5 Cross-section through the brachial region of the embryo in Experiment 225.
- Fig. 6 Cross-section through the posterior part of the wing region in Experiment 179.



Fig I



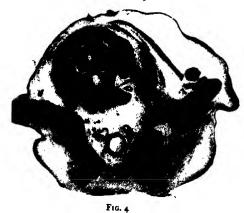
F1G. 2



Fig. 3



F16. 5



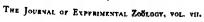
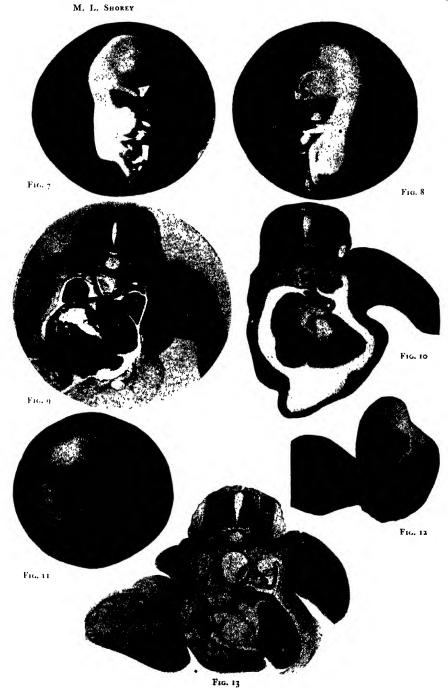




Fig. 6

#### PLATE II

- Fig. 7 External view of the embryo in Experiment 225. This embryo was ectopic, the body wall as well as the wing having been injured by the operation.
  - Fig 8 External view of the same embryo, normal side.
  - Fig. 9 Cross-section through the brachial region of the embryo in Experiment 404.
  - Fig. 10 Cross-section through the operated region in Experiment 60.
  - Fig. 11 External view of the normal side and, Fig. 12, of the operated side in Experiment 706.
  - Fig. 13 Cross-section through the brachial region in Experiment 142.

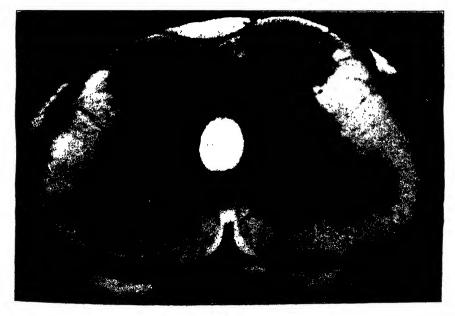


#### PLATE III

- Fig. 14 Cross-section of the spinal cord in the region of the 15th nerve of Experiment 538. The inner margin of the motor nucleus is marked on each side by a cross. The length of the posterior horn from A to B is also decreased on the operated side.
- Fig. 15 Cross-section of the spinal cord in the operated region of Experiment 206. Inner margin of motor nucleus marked by a cross.



F16. 14

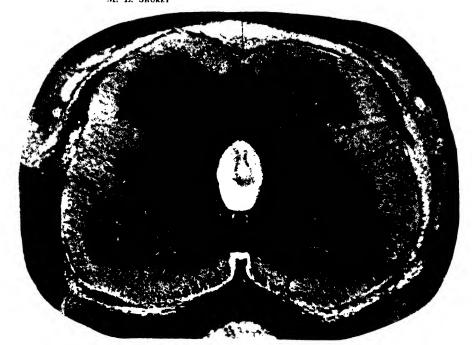


F16. 10

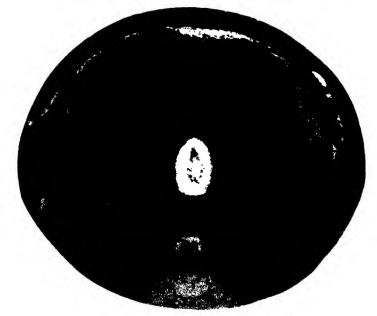
#### PLATE IV

Fig. 16 Cross-section of the spinal cord in the region of the 16th nerve, Experiment 225. Inner margin of the motor nucleus marked by a cross. Operated region is represented on the right in this figure.

Fig. 17 Cross-section representing the typical condition of the spinal cord between the wing and the leg in Experiment 225. Operated region on right.



F10. 16

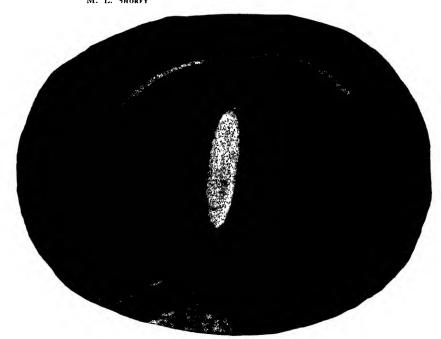


F1G. 17

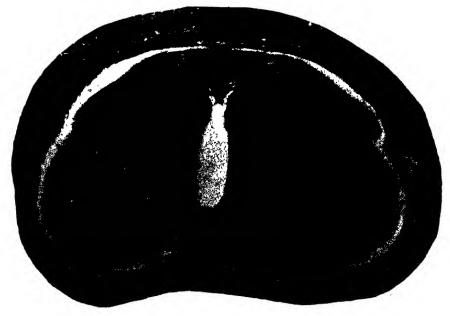
#### PLATE V

Fig. 18 Cross-section of the spinal cord at the level of the 15th nerve in Experiment 179. Inner margin of motor nucleus marked by a cross.

Fig. 19 Cross-section of the spinal cord in Experiment 179, typical of the condition between the wing and the leg of this embryo.



Frc. 18

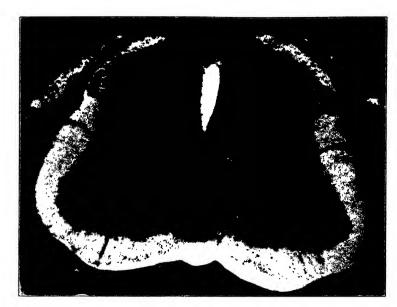


F16. 19

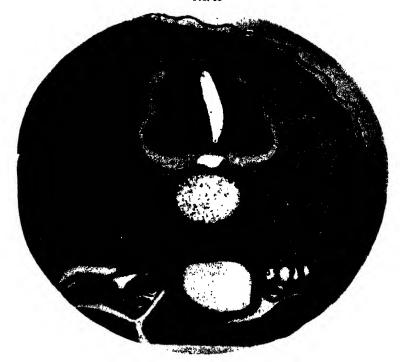
#### PLATE VI

Fig. 20 Cross-section of the spinal cord at the level of the 15th nerve in Experiment 324.

Fig. 21 Crost-section through the brachial region of Experiment 706. The myotome, ganglion and nerve trunk are missing on the operated side. The clear space left apparently by the migration of the ganglion cells to unite with the ganglion below is marked by a+.



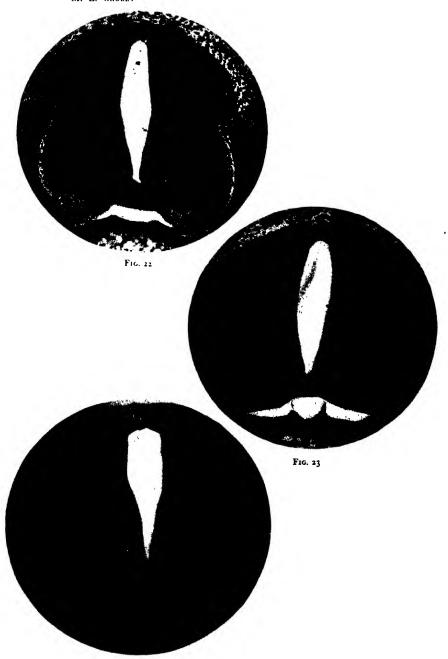
F16, 20



#### PLATE VII

Fig. 22, 23 Cross-sections of the spinal cord at the level of the 15th nerve in Experiments 404 and 79, respectively.

Fig. 24 Cross-section of the spinal cord in the operated region of Experiment 142.



F16. 24

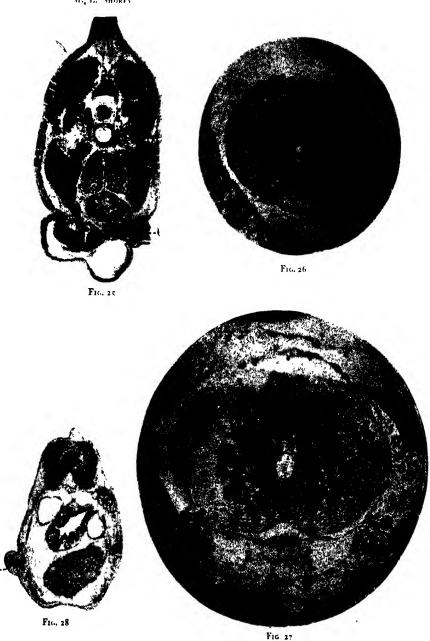
#### PLATE VIII

Fig. 25 Cross-section of Amblystoma tigrinum, Experiment 25, 33 days after the first removal of the limb bud. 1, regenerating limb.

Fig. 26 Cross-section of the spinal cord 10 days after removing the limb in Amblystoma tigrinum. v, ventral horn in operated side.

Fig. 27 Cross-section of the spinal cord of Amblystoma tigrinum, 33 days after the first removal of the limb bud in Experiment 25.

Fig. 28 Cross-section through the operated region in Experiment 27, Amblystoma tigrinum.



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#### PLATE IX

- Fig. 29 Cross-section of the spinal cord in Experiment 27 more highly magnified. v, ventral horn region on operated side.
- Fig. 30 Cross-section of the spinal cord nine days after the operation in a specimen developing parallel with Experiment 27.
- Fig. 31 Cross-section through the operated region two months after the removal of two somites in Rana pipiens.
  - Fig. 32 Spinal cord of same embryo more highly magnified.



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#### PLATE X

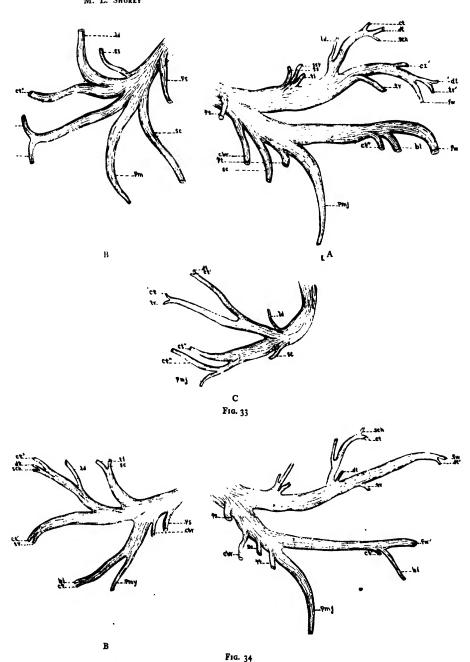
Fig. 33A Peripheral distribution of the brachial plexus on the normal side of Experiment 538.

bt biceps ps pectoralis secundus
cbr coraco-brachialis pt pectoralis tertius
ct, et,' ct'' cutaneous branches sc subclavius
dt, dt' deltoides h scapulo-humeralis
fw, fw' fore wing ss supraspinatus
ld Latissmus dorsi ti teres et infraspinatus
p mj pectoralis major tr, tr' triceps

- B. Peripheral distribution of the brachial nerves on the operated side of Experiment 538. The nerves are labeled as in Fig. 4.
- C. Distribution of the brachial nerves on the operated side in Experiment 206. Lettering as in Fig. 4.

The nerves of the normal side were identical both in size and distribution with those in Experiments 538, Fig. 33 A.

Fig. 34 A Peripheral distribution of the brachial nerves of the normal and, B. of the injured side, in Experiment 225. Nerves lettered as in Fig. 4.



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#### PLATE XI

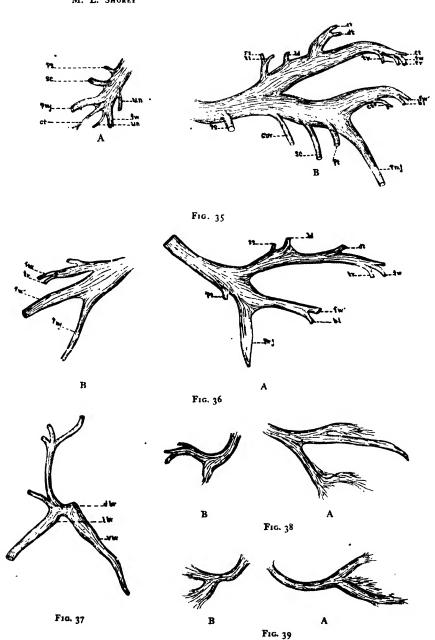
Fig. 35A Peripheral distribution of the brachial nerves on the injured side and, B, on the normal side in Experiment 179. Lettering as in Experiment 538. Un, muscles unidentified.

Fig. 36A Distribution of the brachial nerves on the normal side and, B, on the operated side of Experiment 324. The nerves which are not lettered innervate areas which are not yet sufficiently differentiated to be identified with certainty.

Fig. 37 Typical distribution of each of the six nerves between the wing and the leg of the chick. d br, dorsal branch; l br, lateral branch; v br, ventral branch.

Fig. 38A . Brachial plexus of the normal side and, B, of the injured side in Experiment 404.

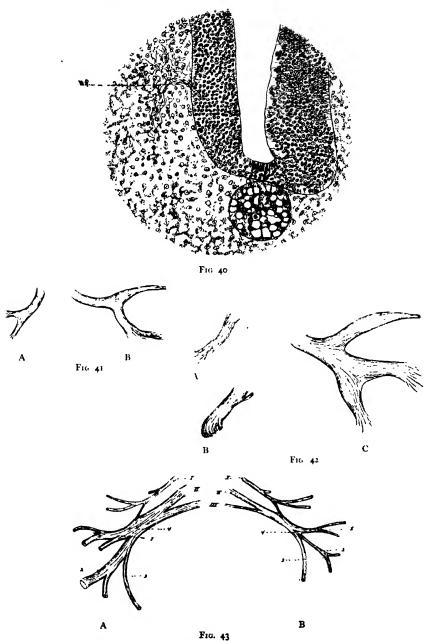
Fig. 39A The brachial plexus of the normal and, B, of the injured side in Experiment 142.



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#### PLATE XII

- Fig. 40 Spinal cord and nerve fibers on the operated side of the second brachial somite in Experiment 69.
  - Fig. 41A Brachial plexus on the normal side and, B, on the operated side of Experiment 60.
- Fig. 42A First brachial nerve and, B, the second brachial nerve on the operated side of Experiment 706.
  - C. Brachial plexus on the normal side of Experiment 706.
- Fig. 43A Sciatic plexus of Amblystoma tigrinum, Experiment 25, normal side, 33 days after the first removal of the limb bud.
  - B Sciatic plexus of the operated side of the same specimen. For explanation, see text.



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# FACTORS OF FORM REGULATION IN HARENACTIS ATTENUATA

### 11 ABORAL RESTITUTION, HETEROMORPHOSIS AND POLARITY

BY

#### C. M. CHILD

#### WITH TWEIVE FIGURES

Changes in polarity, including both the so-called reversal of polarity and other changes occur very readily in Harenactis, probably more readily than in any other actinian which has thus far been made the subject of experiment. The present paper is chiefly concerned with the description and discussion of these changes.

#### I THE REGIONAL FACTOR IN ABORAL RESTITUTION

# I Aboral Restitution in the Esophageal Region

The method of closure of aboral cut surfaces within the œsophageal region is the same as that of oral surfaces in the same region (Child, '09b), i. e., the contraction of the parts injured by the operation approximates the cut margins of body-wall and œsophagus and union occurs. The consequence of this method of union is that the œsophagus opens aborally to the exterior instead of into the enteron, and no connection exists between the enteron and the exterior. In this respect Harenactis resembles Cerianthus and all other species of actinians which I have examined.

The further history of such esophageal pieces depends to a considerable extent upon their length; short pieces from the oral part of the esophageal region—e. g., distal to the line a in Fig. 1—usually do not live for more than a week or two and the regulatory changes at the aboral end rarely proceed beyond the closure of

the wound. Longer pieces—e. g., distal to the line b or between the lines a and b in Fig. 1—may live for several weeks and in such pieces tentacles very often appear at the aboral end.

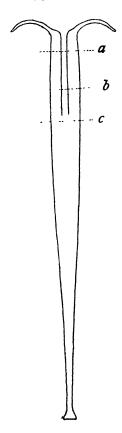


Fig. 1 Diagrammatic outline of Harenactis, indicating levels of section.

But partial isolation of some portion of the œsophageal region by a lateral incision extending only part way through the body is a more satisfactory method for the production of heteromorphic tentacles than complete isolation. After partial isolation the part in question lives indefinitely and in every case that I have observed gives rise to heteromorphic tentacles. For convenience the two methods and their results are considered separately.

# Complete Aboral Discs in the Œsophageal Region

Pieces from the esophageal region with a cut surface at each end often contract irregularly or so strongly that the œsophagus is more or less completely extruded from one end and closure is delayed or prevented: otherwise these pieces give the same results as those with cut surface at the aboral end only. In order to avoid these irregularities pieces including the original oral end of the animal and the old tentacles may be used, e. g., pieces including all of the region distal to the level b in Fig. 1. Such æsophageal pieces show a much higher death rate than pieces of equal size from other regions of the body. In my experiments 50 per cent or more of these pieces died without producing aboral tenta-Pieces that remain in good condition for a week or more usually produce aboral tentacles. These tentacles arise in the same manner as oral tentacles, but less rapidly. In general when a given level of the body, e. g., the level b in Fig. 1, forms the oral end of a piece, tentacles appear much more rapidly than when the same level forms the aboral end of a piece. At the level b, for example, oral tentacles usually became visible in my experiments about forty-eight hours after section, while aboral tentacles appear after six days or more.

The heteromorphic tentacles may reach a length of eight to ten millimeters and would undoubtedly become longer, were it not for the fact that the distension in these esophageal pieces gradually decreases until they are completely collapsed, after which death soon occurs. As the distension decreases the original oral tentacles undergo gradual decrease in size and atrophy at the tips until they are the same size as the aboral tentacles: in later stages the aboral tentacles also undergo decrease and atrophy as the distension decreases still further, until both oral and aboral tentacles may be reduced to short stumps. In general the history of these esophageal pieces is similar in Harenactis and Cerianthus (Child, '04) except that in Cerianthus heteromorphosis has been observed only very rarely (Child, '05).

Fig. 2 shows a diagrammatic longitudinal section of an œsophageal piece with heteromorphic tentacles. At the stage figured the oral tentacles are undergoing atrophy at their tips, but the smaller aboral tentacles are still intact.

The ability to produce tentacles at the aboral end exists throughout the œsophageal region of Harenactis. Every piece from this region which does not collapse and die within the first few days after section produces them. Their number and relation to the mesenteries is the same as at the oral end; in fact an aboral disc thus formed can scarcely be distinguished after a few days from an oral disc, except by the smaller size of its tentacles.

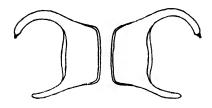


Fig. 2 Œsophageal piece (between lines a and b, Fig. 1) in section with oral and heteromorphic tentacles.

## Partial Aboral Discs in the Œsophageal Region

Partial isolation of some portion of the esophageal region from the regions aboral to it is readily accomplished by a lateral incision involving both body-wall and esophagus on one side (Fig. 3). This is the method employed for the production of lateral partial discs in Cerianthus (Child, '05), and partial discs arise in the same manner in Harenactis.

The course of restitution in these cases is briefly as follows: The contraction following the operation approximates the cut margins of œsophagus and body-wall above and below the cut and union occurs as indicated in Fig. 4, leaving a new lateral opening into the œsophagus. Within two or three days tentacles begin to develop proximal to this opening, their number being determined by the number of intermesenterial chambers cut across by the incision: thus far these cases show the same results as Cerianthus. A few days later, however, tentacles also begin to develop distal to the opening, i. e., at the aboral end of the partially isolated œsophageal region (Fig. 5). When the incision lies proximal to the level of the mesenterial ostia these heteromorphic tentacles

may attain the same length as the others (Child, '09a), otherwise both they and the tentacles directly distal to them at the original oral end undergo gradual atrophy and the whole strip finally disappears as in Cerianthus.

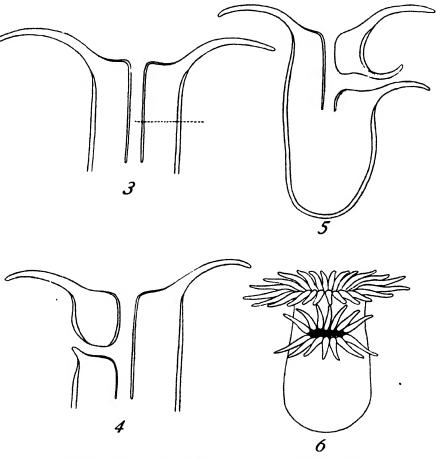


Fig. 3 Oral region of body in longitudinal section: dotted line indicates the operation for producing lateral mouths.

In these cases then a partial aboral disc is formed distal to the lateral opening and a partial oral disc with the same number of tentacles—except in cases of irregularity of closure—develops

Fig. 4 Early stage after the operation indicated in Fig. 3.

Fig. 5 A later stage, showing heteromorphic tentacles above the lateral opening.

Fig. 6 Surface view from side, showing lateral mouth with normal tentacles below and heteromorphic tentacles above.

proximal to the opening. Fig. 6 shows an individual with oral and aboral partial discs after the tentacles have attained complete development.

These heteromorphic discs developed in every case—some twenty-five—where lateral incisions involving both œsophagus and body-wall were made.

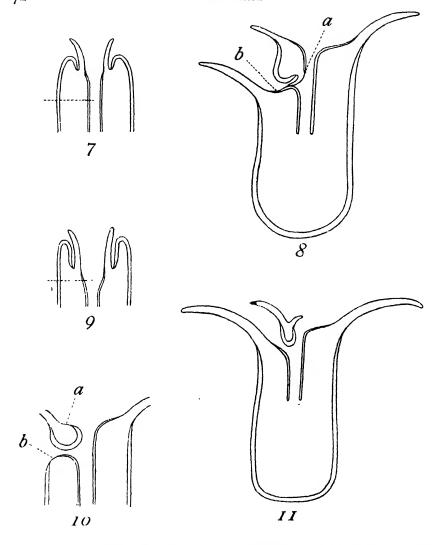
Certain interesting modifications of the results occur when the lateral incisions are made in animals with the oral end more or less completely invaginated. These cases often occur against the will of the experimenter, for when the animals are first brought into the laboratory they are extremely sensitive and a very slight stimulus is sufficient to bring about invagination. Consequently it often happens that more or less invagination has occurred before the scissors reach the œsophagus. Two cases will serve to indicate what may occur under these conditions.

In the first of these cases the relation between the incision and the œsophagus and body-wall was approximately that indicated in Fig. 7. The œsophagus and disc had undergone a certain amount of invagination before the incision reached the œsophagus, consequently the portion of the esophagus distal to the incision is shorter than the portion of the body-wall, as the figure shows. Union between œsophagus and body-wall above and below the incision occurred as usual in this case, but the regions of union occupy positions different from those in the preceding cases. Distal to the cut the body-wall and cosophagus unite at a, Fig. 8, i. e., at a point facing the lumen of the œsophagus when the animal is extended. Proximal to the incision the union occurs at b, Fig. The results are exactly the same as if a lateral incision extending obliquely toward the distal end of the body had been made while the animal was fully extended. The new tentacles arise as usual in relation with, but a short distance from the line of union both above and below the incision. Consequently the tentacles below the incision, i. e., the oral tentacles on the proximal portion. arise somewhat further proximally than in the preceding cases and the partial disc is somewhat oblique (compare Fig. 8 with Figs. 4 and 5). In later stages this obliquity gradually disappears in consequence of growth of the body-wall proximal to these tentacles.

In the region distal to the incision, however, the new heteromorphic tentacles arise in the lumen of the œsophagus (Fig. 8). It is evident from the figure that their origin at this point does not constitute a change from the usual relations of tentacles to the line of union. They arise at or rather very near the aboral end of this partially isolated region of the body-wall exactly as in other cases described (Figs. 2 and 5). In cases of this kind the tips of these aboral tentacles can often be seen protruding from the lateral mouth when the animals are fully extended and distended, though they sometimes extend down the œsophagus and are not visible at all from the exterior. Under these conditions the heteromorphic tentacles do not usually attain any great length. This failure to develop is undoubtedly due to the fact that the pressure of other parts upon them prevents their distension and consequent growth. When the animal is distended the region proximal to the incision presses upon the aboral end of the distal region and the walls of the œsophagus are usually more or less closely applied to each other, so that conditions for the growth of tentacles are very unfavorable. In a number of cases I have seen these aboral tentacles gradually crushed out of existence.

The second case of this kind to be described is shown in Figs. 9 to 11. Here invagination has proceeded so far before the incision was completed that the disc instead of the œsophagus was involved (Fig. 9). Fig. 10 indicates the relations of parts after union had occurred and the animal had become distended again. Distal to the incision the cut aboral surface of the body-wall had united with the cut surface of the disc so that the line of union was at a (Fig. 10). Proximal to the incision union between body-wall and œsophagus occurred at b.

As in the preceding case the tentacles appear in relation to the cut surfaces of the body-wall, but in the case of the oral partial disc proximal to the incision regulation gradually brings the parts into the usual positions (Fig. 11). In the region distal to the incision, however, the heteromorphic tentacles arise between the old tentacles and the mouth (Fig. 11) on what is apparently the disc, so far as its position is concerned, though it is actually the aboral end of the partially isolated region of the body-wall, as is



Figs. 7 to 11 Lateral operations and their results in partially invaginated animals: in longitudinal section.

- Fig. 7 Operation on slightly invaginated animal.
- Fig. 8 Result of operation: the heteromorphic tentacles are located at a, within the œsophagus.
- Fig. 9 Operation on individual more completely invaginated.
- Fig. 10 Early stage following operation of Fig. 9: a and b indicate the regions of union of cut margins of body wall and œsophagus.
- Fig. 11 Later stage of same individual: heteromorphic tentacles appear on the upper surface near the original oral tentacles.

evident from Fig. 10. In all cases of this kind both the old tentacles distal to the incision and the new aboral tentacles undergo atrophy and the whole region disappears because the mesenterial ostia are always proximal to the incision, and this region, therefore does not share in the distension of other parts of the body (Child, '09a). The atrophy at the tip of the old tentacles is shown in Fig. 11.

All possible gradations between the condition represented in Fig. 11 and the usual condition (Figs 4 and 5) can be obtained according to the degree of invagination existing at the time the incision is completed. Attention may again be called to the fact that in all such cases the new tentacles appear in the same relation to the cut surfaces, though their space relations to other parts may differ widely.

## 2 Aboral Restitution in the Subasophageal Region

Under certain special conditions to be described in a later section heteromorphic tentacles may appear in regions aboral to the œsophagus, but where the closure of the wound occurs in the usual manner, i. e., by approximation of the parts of the aboral cut surface of the body-wall and the formation of new tissue between them, as described in the preceding paper, tentacles have never been observed at the aboral end.

Even when the level of section is immediately proximal to the cesophagus (c, Fig. 1) closure occurs to form an aboral end, at least so far as appearance is concerned. Æsophagus and bodywall never unite with each other unless a cut surface is present on each: moreover, these two surfaces must be connected by continuous cut margins of mesenteries in order that such union may occur. These conditions exist only when the section involves the cesophagus, never when the level of section lies aboral to the cesophagus.

Section at any level proximal to the esophagus is followed by closure of the aboral end, though the closure may be more or less delayed by protruding muscles and mesenteries. No outgrowth of a new aboral end such as is found in Cerianthus solitarius

(Child '03, pp. 257 to 259) ever occurs, but the area of new tissue enlarges somewhat as the body becomes distended after closure. The actual restitution of the aboral portions removed is accomplished, so far as it occurs, by the gradual growth and redifferentiation of the more proximal portions of the piece.

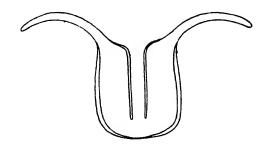


Fig. 12 Restitution in a piece including the whole esophageal region.

The question as to whether complete aboral restitution occurs at all levels of the body requires some consideration. Fig. 12, for example, represents the distal piece resulting from section of the body at the level c, Fig. 1. In this case practically the whole region containing the retractor muscles has been removed and the piece represents little more than the œsophageal region. Such pieces may live for months without food, though they of course decrease in size. During this time the œsophageal region increases somewhat in length, but never attains anything like the usual proportions, at least never within the four and a half months over which my observations extended. Restitution of the retractor muscles in such individuals does not occur to any great extent and they remain wholly incapable of invagination. Moreover, I have never observed attachment to the substratum by the aboral end in these cases.

There can be little doubt that the absence or small amount of aboral restitution in these cases is, at least in large part, the consequence of the conditions of existence. In an earlier paper (Child, '08b) I described certain regulatory changes in shape and structure which occurred when the animals were kept for long

periods without sand in which to burrow. Briefly stated, these changes consist in a very great decrease in length with considerable atrophy of the more proximal regions, including the retractor muscles. Pieces undergoing regulation were kept under the same conditions, and since under these conditions reduction and atrophy of the more proximal portions of the body occurs to a considerable degree in unmutilated individuals it cannot be expected that restitution of these parts shall be complete. In short the condition of physiological equilibrium differs widely from that existing under the usual conditions in nature, i. e., when the animal is in its burrow in the sand. Consequently restitution under these altered conditions consists in an approach to the new condition of equilibrium determined by these conditions, not in an approach to the original condition of equilibrium determined by conditions which no longer exist. In such cases as Fig. 12 then, restitution is approximately complete for the conditions under which the individual is living, though it is not complete with reference to the animal as we usually find it in nature.

There is nothing inherent in Harenactis which determines the restitution of these missing parts irrespective of the environmental conditions. As regards certain features, at least the form and structure of the species are the product of its constitution and the environment and with respect to these same features the same is true for restitution. Under the conditions of the experiments in question there is not and cannot be any stimulus for the restitution of the proximal regions of the body in the form characteristic of the species in nature, since these conditions determine reduction and partial disappearance of these parts in individuals which originally possessed them.

In Cerianthus solitarius (Child '03, etc.) the shape and structure of the body are much less dependent upon external conditions, though not wholly independent of them, and there removal of a proximal region of the body is followed by at least partial restitution. Cerianthus æstuarii (Child '08a) occupies a position intermediate between C. solitarius and Harenactis in this respect. When kept without sand marked decrease in length occurs and the more proximal portions undergo some degree of reduction, and

correspondingly restitution at the aboral end in pieces kept under the same conditions does not proceed much beyond closure of the wound, though when the animals are allowed to burrow in sand it is much more nearly complete.

In my experiments with Harenactis I endeavored to place pieces like Fig. 12 under natural conditions, i. e., I embedded them in sand with only the oral ends protruding, but their movements always resulted in their gradual emergence from the sand. Their emergence was due to the fact that since the aboral end was not firmly fixed in the sand contraction often drew the aboral end upward instead of the oral end downward and the sand gradually filled the space beneath the body. Longer pieces are more likely to remain in the sand since the more proximal portions are less likely to be dislodged by the contractions. I have no doubt, however, that if these shorter pieces can be kept in the sand aboral restitution will at least proceed much further; in all probability they will attain the usual form of the species. I hope to obtain further data on this point at a future time.

As noted above aboral restitution in the subæsophageal region does not show any marked regional differences. So far as I can determine, the amount of redifferentiation is in some degree proportional to the size of the portion removed, i. e., short pieces like Fig. 12 representing the more distal portions of the body proceed somewhat further in the restitution of the proximal parts than do longer pieces. If restitution consists in an approach to the condition of physiological equilibrium characteristic for the existing conditions, as I believe it does, this difference is to be expected, for when we remove the whole subæsophageal region we remove some portions which are characteristic of the condition of equilibrium under the experimental conditions.

One regional difference in aboral restitution does occur, however, though, macroscopically it is physiological rather than morphological. This difference concerns the functioning of the aboral end of the piece as a foot. As I pointed out in an earlier paper (Child '08b) the region of attachment shows no marked differences in structure from other regions of the body-wall. As a matter of fact, however, the more proximal the level of section the more often does attachment of the aboral end thus formed occur. Pieces like Fig. 12 have never been observed to attach themselves and often pieces comprising almost the whole length of the body do not, but my notes record the occurrence of attachment more frequently in longer than in shorter pieces. The question of the localization of the power of attachment was discussed in my earlier paper (Child '08b, p. 11): I need mention here only that it is more marked toward the proximal end and that its limits apparently differ more or less widely according to conditions.

# 3 Analysis of the Experimental Data with Reference to the Regional Factor

The facts are briefly as follows: at all levels within the œsophageal region restitution from an aboral cut surface results in the formation of structures normally characteristic of the oral end; when the operation involves the whole cross-section of the body a complete disc with twenty-four tentacles is formed similar to that at the oral end, and when incisions involving only a part of the circumference are made partial discs with tentacles corresponding in number to the number of intermesenterial chambers cut across by the incision are formed. At all levels proximal to the œsophageal region aboral restitution results in the formation of structures normally characteristic of the aboral end, though environmental conditions may prevent complete restitution of the parts removed. The formation of heteromorphic structures is then limited to the œsophageal region in the cases under consideration.

This definite localization of heteromorphosis may apparently be determined by one or both of two possible regional factors or factor-complexes, viz: first, the physiological character or constitution of the œsophageal and subæsophageal regions and, secondly, the method of closure of the aboral end in the two regions. My experiments show clearly that external factors are not concerned.

As regards the first of these factors, the physiological character or constitution, it is evident that the formation of heteromorphic discs and tentacles would be impossible if the parts concerned did not originally possess or acquire after isolation a certain definite reactive capacity. The question as to whether this capacity is an original possession of the parts in question or whether it is acquired after isolation leads at once to the consideration of the second factor, viz: the method of closure, since this is, so far as can be determined, the only other regional factor possessing the same localization as the heteromorphic reaction.

The method of closure in the œsophageal region is always the same at both oral and aboral ends (Child, 'oob) and consists in the union of the cut surface of the body-wall with that of the œsophagus, so that the œsophagus does not connect with the enteron but opens externally at each end. In the preceding paper attention was called to the fact that this method of closure follows necessarily from the structural relations of parts in the esophageal region and the character of the wound reaction, which consists in the contraction of all parts involved. This method of closure brings the various parts at the two ends of the piece into similar space relations and into similar relations of organic continuity with each other, and it seems at least possible that the establishment of these conditions in two complexes of similar parts may be a factor in determining that the two complexes shall react similarly. Under these conditions the correlations between the parts in each complex must be similar, consequently a similar constitution being postulated, similar reactions are to be expected. On the other hand, the establishment of similar relations of space and organic continuity in two complexes can scarcely be expected under similar external conditions unless the two complexes consist of similar In short, since the two ends of an œsophageal piece of Harenactis are very similar in structure and behave similarly under like conditions we are forced to the conclusion that they are essentially similar physiologically. In restitution their structural and physiological similarity determines that closure of the wound shall occur in the same manner, this method of closure in turn determines that similar relations of space and organic continuity, i. e., similar physiological correlations, shall be established, and these finally determine the occurrence of similar reactions, i. e., in the present case the restitution of similar structures.

Clearly then the experimental evidence leads us to the conclusion that no essential qualitative axial, i. e., polar, differences in constitution exist in the esophageal region of Harenactis. titative axial differences undoubtedly do exist, as is shown by the fact cited in the preceding paper (Child, 'oob) that even in the æsophageal region the rapidity of tentacle-formation at the oral ends of pieces decreases with increasing distance of the level of section from the original oral end. Moreover, aboral tentacles appear much later than oral tentacles at the same level, as was noted above in the present paper. This retardation of aboral tentacles as compared with oral at the same level cannot be due to preëxisting and persisting quantitative axial differences, since it appears only in cases of heteromorphosis, but must be the result of some factor which is present only when heteromorphosis occurs. It is not then, properly speaking, a regional factor and therefore does not concern us at present; as a matter of fact it is undoubtedly a correlative effect, i. e., the presence or formation of oral structures at the oral end of a piece retards the formation of similar structures at the aboral end of the same piece.

The experiments do not afford the slightest evidence for assuming the existence of a directive polar organization in the œsophageal region of Harenactis such as Driesch and certain others have postulated, neither do they show any essential qualitative regional or axial differences in reaction; purely quantitative differences, i. e., differences in the rapidity of reaction, are the only differences which appear, and it is probable that they constitute the only polarity which exists in this region.

In the subæsophageal region, on the other hand, the two ends of the piece react differently, disc and tentacles appearing only at the oral end, while the aboral end redifferentiates more or less completely into the aboral structures removed. There can be no doubt that physiological differences exist at the two ends of the pieces in these cases, but such differences must be differences of correlation rather than of constitution, since any given level of the body in this region except the extreme aboral end gives rise to oral or aboral structures, according as it is in correlation with more proximal or more distal parts. Quantitative differences of con-

stitution do, however, exist in this region: the decreasing rapidity of disc and tentacle-formation and the increasing ability of the aboral end to function as a foot with approach to the original proximal end are indications of such quantitative differences.

The data of oral restitution in the preceding paper show only quantitative regional differences, except at the extreme proximal end of the body: the data of aboral restitution considered above show only quantitative differences, except in the distal, i. e., the œsophageal region. These results are, however, seemingly contradictory, for a given region of the body cannot be qualitatively like and different from other parts at the same time. Evide tly some other factor besides the constitution of the body at different levels is involved in the phenomena of polarity. If we regard this factor as directive, as determining that oral structures shall arise in one direction, aboral in the other, we fall into new difficulties, for the phenomena of heteromorphosis then require a special hypothesis. I believe we must consider the physiological correlations between parts as an essential factor in determining the phenomena of polarity. In the following general consideration of polarity a brief analysis of the factors and the rôle played by each is attempted.

## II POLARITY IN GENERAL

# I The Facts of Regulation and Their Significance

The most conspicuous fact among the data of regulation, so far as they concern polarity, and one might add, among the phenomena of development, is the appearance of characteristically different structure-complexes at the two ends of the principal axis. In regulation this apparent polarization can be well illustrated by the formation of structures typical of one end or the other from cells at exactly the same level of the body, according as these cells form one end or the other of the piece. Such polar differences occur throughout the middle region of Harenactis, Cerianthus and in many other forms. They are of course merely an expression in the isolated piece of what we find in the whole in normal development. These facts alone seem to point to the existence of a

directive organization along the polar axis as the basis of polarity, an organization of the ultimate particles or elements of the protoplasm, such as Driesch assumes. But I believe that these facts alone afford not only an inadequate, but an incorrect conception of the nature of physiological polarity so-called. In order to understand the organization with reference to the "polar" axis it is necessary to consider all the essential facts which indicate difference in capacity of any kind, quantitative or qualitative, along the axis. In addition to the qualitative differences which commonly occur at the two ends of the polar axis, there are three other groups of facts which must be considered before we can safely draw any conclusions with respect to the nature of polarity. These are as follows: First, in many forms the regulation of isolated pieces from regions near the oral or anterior end, and in some cases of pieces from the aboral or posterior regions, does not show the characteristic qualitative differences at the two ends of the axis. In such pieces the structures formed at the two ends are alike, except perhaps in size and time of appearance. These phenomena are commonly termed axial heteromorphosis. In the œsophageal region of Harenactis, for example, the formation of tentacles at the aboral end of the piece is just as characteristic as is the formation of tentacles at one end and a foot at the other in pieces from the subcesophageal region. Similar axial heteromorphosis has been observed in various other coelenterates: in Planaria maculata and P. dorotocephala heads frequently appear at both ends of short pieces from the region near the old head and the region of fission, where the head of the new zooid is at least physiologically specified, and in P. simplicissima, which does not undergo fission, heteromorphic heads appear in short pieces from near the anterior end (Morgan '04). Numerous other cases have been noted.

In some species we find also that in regions posterior to a given level aboral or posterior structures are formed from the oral or anterior as well as from the aboral or posterior end. The formation of tails at the anterior ends of pieces from the posterior half of the earthworm (Morgan '99), and of short posterior pieces of Planaria simplicissima (Morgan '04) will serve as examples.

Evidently then the heteromorphic formation of the structures

characteristic of either pole of the axis may occur, but heteromorphic development of oral or anterior structures is probably of more frequent occurrence than aboral or posterior heteromorphosis; but this is probably due, however, to the fact that the oral or anterior region is a region of greater physiological activity than the other, i. e., negative results in aboral or posterior pieces are often due rather to lack of energy than to anything else.

In general heteromorphosis of oral or anterior structures occurs in regions near the oral or anterior end, heteromorphosis of aboral or posterior structures nearer the opposite end. In certain cases, however, as in Tubularia, axial heteromorphosis of oral structures may be a frequent or characteristic result at most or all levels of the body. Here and in all cases of this kind the external conditions after isolation are a factor in determining the result, consequently such cases do not afford data which can be used directly for the analysis of polarity (Child '07). They tell us nothing about an original polarity except that, if it ever existed, it is no longer present. In fact, as I pointed out in my discussion of polarity in Tubularia (Child '07e) we must distinguish two kinds of axial heteromorphoses, primary and secondary. Primary heteromorphoses are the necessary result of the axial organization of certain regions which existed in the original organism. They occur in all cases where the piece is so short axially that axial differentiation or specification is almost or wholly absent. In the absence of the original organization the parts cannot be correlated with each other as are the parts of the whole. The two terminal regions, being physiologically alike or nearly so, react similarly to the existing conditions, with little or no relation to each other, consequently new specifications are established with reference to these two regions instead of one of them, and we say that the polarity of one end has been reversed. The heteromorphic discs and tentacles in œsophageal pieces of Harenactis are undoubtedly primary heteromorphoses, i. e., there are no marked axial or polar physiological differences in this region in the original animal, and when a piece is isolated new polarities arise between each end and the middle, since conditions must necessarily be different in these two regions. Similarly the posterior heads in short pieces of

Planaria are primary heteromorphoses, also the double hydranths or partial structures in short pieces of Tubularia (Child '07c). Theoretically primary heteromorphosis may occur at any region of the body if sufficiently short pieces are isolated; whether it will actually occur or not will depend upon whether the pieces possess sufficient energy to produce morphogenic results.

Secondary heteromorphoses occur when the original axial specification of the organism is present in the piece at the time of isolation, but cannot persist under the conditions of the experiment. Under certain conditions secondary heteromorphoses occur in Harenactis, but these will be considered in a following paper. In Tubularia the formation of aboral hydranths in long pieces of the stem is, as I have shown (Child '07a, '07c, '07e), a secondary heteromorphosis.

In the case of primary heteromorphosis, then, we obliterate or almost obliterate the differentiation or specification in the axial direction by isolating a piece so short that its two ends are almost or quite similar physiologically: in secondary heteromorphosis the original organization persisting in the isolated piece at first is more or less completely obliterated by conditions arising after isolation of the piece. In Harenactis, for example, the union of the oral with the aboral end is apparently a factor, probably the chief factor, in obliterating the original polarity, and thus, so to speak, clearing the way for the establishment of a new polarity.

But the important point for the present consideration is that the phenomena of primary axial heteromorphosis indicate that the polar specification is not uniformly distributed along the axis. In the whole œsophageal region of Harenactis, for example, there is, according to this view, nothing more than a slight quantitative difference at the two ends of the axis of any piece, i. e., polarity in the usual sense of qualitative polar differences is absent or practically absent from this region. If my position is correct the same must be true for any region of any species in which primary heteromorphosis occurs. And finally, in forms like Planaria simplicissima where short pieces from regions near the old head, form heteromorphic heads and short posterior pieces form heteromorphic tails, these two regions must be so widely different in their

physiological specification and capacities that we may call the differences qualitative and not quantitative. In other words the extreme anterior region is capable of reacting only in a particular manner, i. e., so as to produce heads at either or both ends, while the extreme posterior region can produce only tails. In both cases the production of the characteristic structures fails to show any indication of a directive organization: the organization, specification, or whatever we may call the reactive capacity is, so far as the facts go, very clearly regional and direction is determined merely by the relations between the cut surface and other parts. I believe then that the facts justify the conclusion that the terminal regions of the body may differ so widely from each other in their constitution as to be incapable of reacting similarly at any point; consequently when pieces from these regions are isolated they produce similar structures at both ends if they produce anything, and the structures produced are those characteristic of that end of the parent body from which the piece is taken. Very commonly, as in Harenactis, primary heteromorphosis occurs only in the anterior or oral region and short pieces from the extreme proterior or aboral region form nothing. This difference is probably due, however, as was noted above, rather to a difference in energy or available material than to any fundamental difference in organization. There is abundant evidence to support the conclusion that in at least a very large number of forms the anterior or oral pole is primarily the region of greatest formative energy or activity, or of greatest rapidity of reaction.

We cannot then, I believe, escape the conclusion, that qualitative regional differences in the terminal portions of the body constitute in at least certain forms a feature of organic polarity, as well as the qualitative differences which usually appear at the two ends of the axis of the piece. In short the terminal regions of the body may be respectively wholly oral or anterior and wholly aboral or posterior as regards their "potences" and primary heteromorphosis is the necessary consequence of this extreme specification, provided sufficient energy for morphogenic reactions is present.

In addition to these two features of axial specification which we

may call qualitative, certain quantitative features exist. Usually, for example, the rapidity of development or the size of oral or anterior structures decreases as the distance between the level from which they arise and the original oral or anterior end increases, and the same is true, mutatis mutandis, for aboral or posterior structures. As was shown in the first paper of this series, the rapidity of tentacle-formation in Harenactis decreases with increasing distance of the level of restitution from the original oral end: in Tubularia (Child '07c, '07e) and in Planaria maculata and P. dorotocephala (Child '06) similar differences exist, though in these planarians a complicating factor exists in the presence at the posterior end of a region physiologically specified as a new zoöid.

And finally, in cases of primary heteromorphosis quantitative differences between the two ends of the axis often exist, as in Harenactis, where the aboral tentacles always appear later than oral tentacles in an œsophageal piece, and not only that, but later than oral tentacles at the same level Similar differences often appear in Tubularia and Planaria.

Summing up what has been said, we find that the restitutional reactions show four distinct characteristics with respect to the principal axis: first, different reactions may occur at the two ends of the axis of a piece (qualitative polar differences or heteropolar phenomena); second, in cases of primary heteromorphosis the heteromorphic reaction very commonly occurs more slowly than the "normal" one (quantitative polar differences); third, the character of the reactions in primary heteromorphoses may differ completely according as the pieces are taken from one or the other end of the axis (qualitative regional differences); and fourth, in most cases the rapidity or energy of the reaction characteristic of either pole decreases as the distance between the level from which it occurs in a given case and the pole where it originally occurred increases (quantitative regional differences). In considering the problem of what we call polarity we cannot neglect any of these phenomena, for all of them have to do with the axis. If we consider polarity with Driesch as a directive organization we cannot account for primary heteromorphoses, in which the two ends of the axis are alike except perhaps quantitatively. If, on the other

hand, we accept Morgan's hypothesis that polarity is a gradation of substances along the axis, another hypothesis is necessary to account for the most conspicuous feature of polarity, viz: the fact that the same tissues may produce totally different structures according as they constitute one end or the other of the axis.

In short, neither the hypothesis of directive organization nor that of regional gradation alone will account for the characteristic phenomena of polarity in animals, for the very simple reason that polarity possesses both regional and directive features. words there are two internal factors in polarity, constitution and In Harenactis or in Planaria the cells at a given level correlation. form tentacles or a foot, a head or a tail, according to the character of their relation with other parts of the organism, i. e., their correlations. This factor of correlation and the part which it plays in polarity have been recognized by the botanists, and it is certainly no less important in animals than in plants. Whether a given cell complex gives rise to an anterior or posterior, an oral or aboral structure, cannot possibly depend upon itself alone, since it is originally the same in both cases, but nevertheless gives rise to different results.

Morgan's hypothesis can account only for regional, never for polar phenomena. According to his latest statement of the hypothesis (Morgan '07, pp. 378 to 380), a head forms at one end of a piece because there is more head-forming substance there, and a tail at the other end because that contains more tail-forming substance. This amounts exactly to the assertion that heads and tails appear where we find them because they do. Moreover, it affords us absolutely no basis for understanding how either a head or a tail may arise from the same cells according as they form one end or the other of the piece.

On the other hand, Driesch's hypothesis accounts for these differences at the two ends of the axis, but cannot account for the phenomena of primary heteromorphosis. Moreover, the evidence in favor of the hypothesis of a directive organization seems at least unsatisfactory.

I believe that no conceivable hypothesis which concerns constitution alone, or correlation alone, can account for the phe-

nomena of polarity in organisms, for both constitution and correlation, or in other words, both structure and function are factors in determining the character of the phenomena.

I use the term "correlation" in its physiological sense as including all effects of conditions and processes in one part upon another part. It is only within recent years that correlations have become in any real sense objects of investigation. Concerning their nature we learn but slowly: we know that some of them are chemical, others physical, and still others complex, but there seems to be no general recognition among zoölogists such as exists among botanists of their great importance in morphogenesis. I might cite in this connection various misconceptions and misinterpretations of my own work on regulation, for they furnish abundant proof of this fact, but time and changed conceptions of the problems of morphogenesis will perhaps accomplish what discussion cannot.

# 2 Constitution and Correlation in the Phenomena of Polarity

We may say in general that so far as internal factors are concerned, the constitution of a part at any given time determines what reactions are possible in it at that time, and its correlations determine what reactions actually occur. In Harenactis, in Planaria and many other forms the cells of a given level, e. g., the middle of the body, possess a constitution which is capable of giving rise to the structures characteristic of either pole. But whether they actually form tentacles or a foot, a head or a tail in any particular case is very evidently determined by their physiological relations with other parts, i. e., their correlations. Manifestly the cells of a given level must be in a different physiological state when they are in correlation only with parts aboral or posterior to them, from that which exists when they are in correlation only with parts oral or anterior to them. The nature of the differences between these two states we do not at present know, but we cannot doubt their existence. Moreover, both of them are different from the original state when the cells were correlated with both regions of the body.

Formative substances, so-called, do not assist us to explain these facts, they merely state the facts. It is self-evident that where a head is formed there must be "head-forming substances": the real problems lie in the questions of how they come to be there, and why in the same cells "head-forming substances" are active at one time and "tail-forming substances" at another.

My analysis of these qualitative polar differences or heteropolar phenomena in restitution is then as follows: first the tissues involved possess at the time of operation a constitution capable of either reaction; second, their constitution and consequently their reaction is altered in one direction or the other according to the character of their physiological correlations with other parts. These correlations may be either chemical or physical or both; in all probability they are exceedingly complex, as they are known to be in plants.

In the primary heteromorphoses, i. e., in those cases of heteromorphosis where the isolated piece is so short that polar differences are almost or quite absent at its two ends, we have another characteristic restitutional phenomenon. In such pieces the two ends are physiologically almost identical, consequently the correlations between them can have little or no effect in producing a difference between them and any effect must be slight. follows then necessarily that if morphogenic reactions occur at the two ends of the piece they will be almost or quite similar in character. But the constitution of such pieces is different according as they are taken from one or the other terminal region of the original, individual and consequently such pieces may give rise either to heteromorphic heads or tails, oral or aboral structures according to their position in the body. Sometimes, as in the case of Tubularia (Child '07d), the conditions of experiment, in this case the formation of free terminal regions, play a part in determining the character of the reaction, and in various other cases, as in Harenactis, pieces from the extreme aboral or posterior region apparently have not sufficient energy, when isolated, to produce anything. Under these conditions only primary oral heteromorphosis may appear, and this may be limited to the more oral region as in Harenactis, or may occur at any level of the body if the pieces are sufficiently small, as in Tubularia.

But the essential point for our present purposes is that the phenomena of heteromorphosis in short pieces—primary heteromorphosis—are the necessary consequence of the constitution of the material, when the length of the piece is so short that its axial differentiation is reduced practically to zero, and the correlations between parts consequently almost or quite eliminated. But, as pointed out above, the physiological state at the two ends of such pieces must after isolation become different from that at the middle, consequently the two opposed polarities result.

These cases of primary heteromorphosis show us, according to this view, something of the differences of constitution along the axis. In such a case as that of Planaria simplicissima (Morgan, '04), where short pieces from near the old head form double heads and those from the posterior region double tails, it is evident that the two terminal regions of the body are qualitatively different in constitution. Moreover, these phenomena afford in my opinion the strongest evidence against the hypothesis that polarity consists in a directive organization or orientation.

But correlations between the two terminal regions are not entirely absent in cases of primary heteromorphosis. In Harenactis, for example, the aboral tentacles in the œsophageal region always appear, not only later than the oral tentacles, at the opposite end of the piece, but later than oral tentacles at their own level. Evidently there is some slight physiological difference, at least a quantitative one, between the two ends of the piece, for the processes at the oral end retard those at the aboral end. Similar differences between the two ends occur sometimes or always in various other forms. In primary heteromorphosis in Tubularia I found that at least very commonly the aboral primordium or partial primordium was considerably delayed in the earlier stages (Child '07d), though after emergence the two were usually more nearly alike. In Planaria the two heads are often different in size and one may appear before the other.

Here again in the retarding influence of the oral or anterior end upon the aboral or posterior end in short pieces we find a correlative factor of polarity. The fact that the development of the aboral structures is slower than that of oral structures at the same level, i. e., composed of the same cells, shows very clearly that the factor involved is correlative, not constitutional.

Finally we have to consider the quantitative regional differences which appear along the axis in restitution. Harenactis affords a fairly good illustration of such differences. Here the rapidity of tentacle development and to some extent also the size of the tentacles decrease as the distance between the level from which they arise and the original oral end increases: in the extreme proximal region tentacles do not appear at all. Similarly, though less clearly, the ability to form a foot decreases toward the oral end until in the œsophageal region it does not occur at all. differences appear in all animals in which the phenomena of restitution are not confined to appendages and peripheral parts, except where they are complicated and modified by various other factors, e.g., the intensity of the correlative stimulus, unsymmetrical organization along the axis, cephalization of the nervous system, etc. Discussion of these factors is not necessary here since they do not alter the fundamental fact that quantitative regional differences in restitution do occur at both poles of the axis, which is all that concerns us at present. These diffferences are very evidently expressions of the constitution of different regions. Harenactis, for example, from a level just proximal to the œsophagus tentacles develop rapidly and attain a large size when this level forms the oral end of a piece, but a foot is rarely if ever formed when the same level lies at the aboral end of a piece. At a level near the proximal end of the body tentacles appear slowly and remain small, but a foot forms readily from the same level. Evidently the new correlations established after isolation of the pieces do not completely obliterate the previously existing differences of constitution. If these were the only phenomena of polarity Morgan's hypothesis of a graded constitution would account for the facts.

To sum up: The qualitative regional differences as exhibited in primary heteromorphoses and the quantitative regional differences which appear in the structures of either pole at different levels are determined by the constitution of the material which existed before isolation of the pieces: the qualitative axial or polar

differences, i. e., the heteropolar phenomena and the quantitative axial or polar differences, i. e., the differences between the two ends of the piece in primary heteromorphosis are determined by the correlations existing between parts after isolation. Preëxisting constitution and present correlation are then the two factors or factor-complexes which determine the phenomena of physiological polarity as they exist in regulation.

These two factors are in other words the morphological and physiological aspects of the problem of polarity. I believe that every problem of morphogenesis possesses these two aspects and that any attempt either at purely morphological or purely physiological interpretation must of necessity be incomplete. When we assume the structural basis as given we may say that all problems of morphogenesis are physiological or functional in character, as I have done in certain papers. When we assume the various processes as given morphogenesis becomes a morphological problem. But strictly speaking living structure without function and function without structure are alike inconceivable: changes in structure must accompany changes in function and changes in structure cannot occur without producing changes in function. Recent criticisms of some of my work remind me that it is perhaps necessary, to emphasize once more the fact that I am using the word function in its broadest sense as synonymous with process or activity.

#### III SUMMARY

- In the œsophageal region of Harenactis tentacles and disc are always formed at the aboral as well as the oral ends of pieces, provided the pieces live long enough and become sufficiently distended. The aboral mouth in these cases is not a new formation but is the result of fusion of the cut end of the body-wall with the cut end of the œsophagus so that the latter remains open.
- 2 Complete or partial aboral discs develop in the œsophageal region according as the operation involves the whole cross-section of the body or only a part of it.
  - 3 The formation of aboral tentacles is less rapid than that of

oral tentacles in the same piece and also less rapid than that of oral tentacles from the same level of the body.

- 4 In the subæsophageal region aboral tentacles never appear except under certain experimental conditions to be considered later.
- 5 When only the most proximal regions of the body are removed restitution of a foot region occurs in a short time, but as the portion removed becomes larger the restitution of missing parts becomes less rapid and less complete, until in cases where most of the subæsophageal region is removed, only a slight degree of restitution occurs. Absence or incompleteness of aboral restitution in these cases is undoubtedly due wholly or in large part to the environmental conditions. When the animals are kept in water without sand in which they can burrow a considerable amount of reduction and atrophy occurs and this is greatest in the proximal regions and decreases distally. Under conditions in which these parts undergo atrophy when they are present it is not to be expected that their complete restitution should occur in pieces from which they have been removed. The form and constitution of individuals living in burrows in the usual manner are different from those of individuals living in water without sand, and restitution of the form characteristic of the existing environment does occur in my experiments. If the short pieces could be kept in burrows it is probable that at least much more complete restitution of the form occurring in nature would take place.
- 6 Two groups of factors are involved in the phenomena of polarity as they appear in restitution: these are constitution and correlation. The regulatory phenomena of polarity may be grouped under four heads as follows:
- a Qualitative axial or polar differences in restitution (heteropolar phenomena): the same cells may produce qualitatively different structures according to their position at one pole or the other of the piece.
- b Qualitative regional differences (primary heteromorphosis): short pieces from near one pole or the other, or in some cases both, may produce at both ends structures like those present at the nearest pole of the original individual.

- c Quantitative axial or polar differences: in heteromorphosis the heteromorphic structure is very often delayed in development.
- d Quantitative regional differences: when axial phenomena are not complicated by other factors, the rapidity of restitution and often the size or completeness of the parts decreases as the distance between the level at which they form and the similar pole of the original individual increases.

These phenomena may be complicated and altered by various factors such as greater differentiation or specification of certain parts and consequent decreased power of restitution, the intensity of the restitutional stimuli under different experimental conditions, cephalization of the nervous system, etc.

- 7 The regional differences, both qualitative and quantitative, are determined by the regional differences in constitution existing in the original individual at the time the pieces were isolated. They are the result of the structural polarity which is a regional difference in constitution along the axis.
- 8 The axial or polar differences, both qualitative and quantitative, are determined by the correlations existing between the parts of the piece after its isolation. They are the result of the physiological polarity.

#### NOTE

Since this paper was written the results of Lillie's recent centrifuge experiments on Chætopterus eggs have appeared.¹ Lillie's conclusions require a brief consideration in connection with what has been said above concerning polarity.

The persistence of the original polarity and symmetry in the "ground substance" after centrifuging, and irrespective of the position of the granules, has led Lillie to suggest that organic polarity and symmetry are molecular and essentially similar to the polarity and symmetry of crystals. According to this hypothesis polarity is directive, i. e., it is the result of molecular orientation.

I have endeavored to show above that such a hypothesis cannot account for the regional phenomena along the axis, which are observed in regulation both in young and adult forms. Lillie assumes that these differences are due to secondary factors. As

<sup>&</sup>lt;sup>1</sup> Biol. Bull. rvi, 2, 1909.

a matter of fact, however, in almost all if not all observed cases the directive feature becomes less and less conspicuous in isolated pieces, with approach to one or both ends of the body, and with decrease in the size of the piece, while the regional differences become more conspicuous under these conditions. If the directive feature is the primary feature of polarity it seems strange that it should give place more or less completely to a regional feature under these conditions.

Moreover, Lillie finds various evidences of the existence of a molar structure in the ground substance, which he regards as concentric. It seems at least possible that such a structure may possess axial characteristics which have not yet been recognized. The apparent homogeneity of the ground substance is certainly no argument against the existence in it of a polar structure. In short, Lillie's conclusions do not seem to follow necessarily from the facts and his argument is therefore somewhat unconvincing. Morgan does not seem to have presented in his recent paper<sup>2</sup> any more cogent reasons for acepting this hypothesis. In so complex a mixture of colloids and other substances as protoplasm certainly is, the possible morphogenic factors are so numerous and varied that we are scarcely justified in turning at once to the molecule when direct observation fails us.

It seems to me that the hypothesis rests essentially on negative evidence, so far as the organism is concerned, i. e., in the absence of any visible basis for polarity it is assumed that the phenomena concerned are the result of molecular orientation. The phenomena of fluid crystals are undoubtedly of great interest, but it is by no means certain that there is anything more than a very remote analogy between them and the phenomena underlying organic polarity and symmetry. Such an assumption seems to neglect the fact that protoplasm is not a homogeneous chemical substance nor even a simple mixture. Moreover, it exists in cells and within the cells various localized differentiations may occur which may well be the result of a molar structure of the ground substance. Besides all this restitution may occur in isolated pieces after centrifuging.

<sup>&</sup>lt;sup>2</sup> Biol. Bull., xvi, 5, 1909.

At present it is a far cry from visible differentiation to molecular orientation in protoplasm.

And finally the hypothesis is essentially speculative and the problems involved are practically removed from the field of investigation by its acceptance. Consequently we are justified in accepting it only when the facts leave no better alternative. To accept it on the basis of our present ignorance cannot serve to advance actual knowledge. As a stimulus to future research the hypothesis must affect the skeptic rather than the believer.

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# SOME EFFECTS OF EXTERNAL CONDITIONS UPON THE WHITE MOUSE

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#### WITH FOURTEEN FIGURES

The production of definite modifications in the structure, color or size of animals or plants by artificial changes in the conditions of life has been successfully accomplished over and over again by a large number of investigators. I need only allude to such classical instances as the experiments of Dorfmeister, Weismann and others on butterflies, Schmankewitsch on Artemia, Cunningham on flounders, Naegeli and Bonnier on Alpine plants, or to the more recent work of Tower upon beetles and Beebe upon birds. The fact that such considerable modifications may be produced within the lifetime of an individual by physical or chemical means is in itself interesting if regarded simply as an illustration of the plasticity displayed by many organisms. But when we push our inquiry beyond the merely descriptive plane, we are brought face to face with some of the most fundamental problems of biology. Are these modifications adaptive in their character? Do they in any instance correspond to the features which distinguish one natural species or geographical variety from one another? And finally, do such artificially produced modifications reappear in offspring which have not themselves been subjected to the conditions of the experiment? These are some of the questions which demand an answer from the investigator. They are not so simple and easy of solution as may appear on the surface: the first

<sup>&</sup>lt;sup>1</sup> An Investigation of Evolution in Chrysomelid Beetles of the Genus Leptinotarsa. Carnegie Institution, 1906, pp. 320, pl. 30.

<sup>&</sup>lt;sup>2</sup> Geographic Variations in Birds, with Special Reference to the Effects of Humidity. Zoölogica: N. Y. Zoöl. Soc., Sept. 25, 1907, pp. 41, pl. 6.

because we cannot always declare with confidence whether or not a given character is adaptive; the second, because the average experimentalist is as little conversant with the literature and methods of the systematist as the systematist is with those of the experimentalist; and the last, for reasons so numerous that they cannot even be outlined within the limits of the present paper.

Herein are presented some of the results of an inquiry into the effects of differences of temperature and humidity upon the postnatal development of the white mouse. In all, upwards of 400 individuals have been subjected to the experimental conditions during the past three winters, though it must be confessed that the number of mice in any one series has been relatively small. Due allowance has been made for this fact, however, in considering the probability of the various conclusions which are offered below. As will later be pointed out, differences which are great enough and constant enough are to be regarded as significant, even though the number of individuals is small. And it is scarcely necessary to remind my readers that the statistical treatment of even such small numbers as are here under discussion requires a great amount of extremely tedious work.

It must be acknowledged at once that I have been actuated by ulterior motives in pursuing these experiments. My primary object has been to test the question of the transmission of certain characters, or, more correctly, of their reappearance in the offspring. Thus far, it is true, no satisfactory or at all convincing test of this point has been made by the writer, though the experiments are still far from being ended. Large, obvious, and readily measurable changes have, however, been produced in the generations immediately subjected to the conditions employed. Thus one of the primary requirements for the fulfilment of such a test has been realized. It is not the purpose of this paper, accordingly, to present any evidence in favor of transmission. I shall content

<sup>&</sup>lt;sup>8</sup> Some of the earlier results of this work were presented briefly before the American Society of Zoölogists, New Haven, December, 1907 (abstracted in Science, March 20, 1908.

<sup>&</sup>lt;sup>4</sup> To this number must be added nearly 300 others, the data for which were not available when the present paper was being prepared. A brief mention of this most recent series has, however, been inserted on a later page.

myself with recounting some of the modifications produced during the lifetime of the individual.

## ENVIRONMENTAL CONDITIONS

Early in life, in each experiment, the mice were divided into two lots, one of which was transferred to a room artificially heated,5 the other to a room readily accessible to the winter atmosphere. The source of heat employed was a large steam coil which was ordinarily very effective. The room used throughout most of the work was, however, extremely pervious to draughts of air, and a strong wind from the proper quarter would sometimes bring down the temperature as much as 5 or 10° C. in the course of an hour In the fall and early part of the winter, the steam was available during the daytime only; commencing about January I, it was turned on night and day. The cold room used in the two earlier series of experiments was situated in another part of the same building as the warm one. During much of the time a window was kept open in the former, though this was not necessarv when the wind blew from the right direction, at which times the temperature would fall nearly to that of the atmosphere outside. An unfavorable wind, on the contrary, frequently forced in warm air from other parts of the building. Thus in the case of neither of these rooms did conditions favor the maintenance of a very uniform temperature, and at times the fluctuations were rather violent. Nevertheless the mean temperature throughout the experiments was very much higher in one room than in the other (Fig. 1), and the fluctuations resulted in little if any harm to the animals. Necessarily the humidity likewise differed to an enormous degree. In the cold room, the air was frequently near the saturation point. In the warm room, on the contrary, this

<sup>&</sup>lt;sup>8</sup> Certain rooms in the U. S. Fisheries Laboratory at Woods Hole, Mass., not otherwise in demand during the winter months, were employed for this purpose. The material equipment has been provided almost wholly by the author himself. I must except a considerable number of cages kindly lent by Professor Morgan during the third winter of the work.

<sup>&</sup>lt;sup>8</sup> During the present winter my facilities have been much better. Through the kindness of the director, Dr. F. R. Lillie, I have had the privilege of locating my cold room in a small unheated building belonging to the Marine Biological Laboratory. Here, therefore, the only fluctuations are those due to changes of weather.

same air, being heated to a temperature of 20° or 30° C., its humidity (degree of saturation) fell to 50 per cent or even considerably less. There is a popular fallacy to the effect that moist air may be dried by heating, after the manner of one's damp clothes. The notion contains this element of truth, of course-namely that heating, except when the same degree of saturation is maintained by evaporation, does increase the capacity of the air for taking up moisture from other objects. Accordingly, in the present experiments, the sawdust upon the floor of the cages and the cotton waste used for bedding were much damper in the cold room than in the warm one. Indeed there was commonly no perceptible dampness at all in the latter, while in the former it became distinctly moist if the changing were long neglected. The relative humidity was determined at rather irregular intervals by the use of an ordinary psychrometer or wet-and-dry-bulb thermometer. The percentage values were obtained from the scale attached to the "hygrophant," manufactured by J. S. F. Huddleston of Boston, though the instrument actually used was a similar one of another make. These percentages are probably to be regarded as rather rough approximations. They are, however, believed to suffice for present purposes. The temperature, in each room, was recorded continually by means of a thermograph, and thus the daily and hourly fluctuations of temperature could be followed.7

Since the conditions and methods of treatment differed considerably in the different series of experiments, a further account of these is deferred to the separate discussions of the latter. No account of the feeding nor of the general care of the mice is regarded as necessary here. It is sufficient to state that, except for the differences in temperature and humidity above mentioned, it was my endeavor to maintain all conditions as similar as possible for the two contrasted lots of animals.

<sup>&</sup>lt;sup>7</sup> The laborious task of computing the mean temperatures, etc., from the tracings upon the thermograph sheets has been mainly performed by my wife and my mother.

<sup>&</sup>lt;sup>8</sup> I must acknowledge my indebtedness to Prof. W. E. Castle and Prof. T. H. Morgan for valuable suggestions relative to the care of the mice. The stock was all obtained from Miss Abbie E. C. Lathrop, the well-known animal breeder, of Granby, Mass.

### CHARACTERS CHOSEN FOR MEASUREMENT

In the selection of these characters it was of course desirable to choose one or more which might be supposed to be influenced adaptively under the conditions of the experiment. of hair was therefore chosen as one of the values to be determined. Theoretically the hair might be subjected to quantitative treatment in three different ways: (1) by determining the average length of the hairs on each pelt, based upon a considerable number of individual hairs, so taken as to be representative of the entire lot; (2) by finding the number of hairs on a given unit of skin area; (3) by ascertaining the weight of the entire pelage or hair-coat of each mouse. The first method was very soon found to be impracticable, owing to the fact that the hairs are seldom straight enough for the purpose, being commonly curved or zigzag. The second and later the third methods were, however, employed more or less advantageously. For the determination of the number of hairs in a given unit of skin, the latter, immediately after removal from the freshly killed animal, was subjected to a stretching process which was as far as possible identical for each pelt. Pinch clamps were fastened at six points on the margin of the skin, and to these were attached fine cords which passed outward over pulleys arranged around the skin in a circle. Each cord bore a 100-gram weight. The pelt was thus gently stretched for five minutes, after which a large cork was pressed lightly against its central region, and the skin pinned to it by a circle of 16 pins. The outlying portions of the pelt were now cut away, and the circular area which was left pinned to the cork was allowed to dry. After a variable interval, depending on convenience, a small disk of the skin was cut out by means of a tool devised for the purpose. This consisted of a steel tube, 11 mm. in diameter, sharpened at one end into an edge. In use, it was pressed rather lightly against the inner surface of the skin and rotated until the latter was completely cut through. The resulting disk of skin was fastened to a black tile -hair uppermost, of course-by means of glue. The hairs were now shaved off with a sharp knife and counted under a low power lens. Two disks from symmetrically placed points on the pelt

of each mouse were used, and it was sought to take them from corresponding regions of the skin in the case of every animal. The entire process required much time, and the counting of the hairs proved to be extremely fatiguing to the eyes. Moreover the great individual differences in the density of the hair and even the differences in density on the two sides of the same individual showed that satisfactory figures could not be obtained without making a large number of determinations. For these and for other reasons the counting of hairs was abandoned after the first year's series of experiments. The results, so far as obtained, will be presented later in the paper.

The method of weighing the total pelage of each mouse has proved to be by far the most satisfactory one. Aside from the greater ease and accuracy of technique, this method has the advantage of showing whether or not the total quantity of hair has varied under the different conditions. It has the disadvantage, however, of not showing how the quantity has been affected, i. e., whether by the augmentation of the number of developed hairs, or of their length or their diameter. The process employed was as follows: The pelt after drying was placed in a saturated solution of calcium hydrate, and left for three days (occasionally longer). 10 After rinsing, the hair was scraped off with a moderately sharp scalpel. Most of the hair, in the majority of pelts, could be removed very readily; the remainder sometimes required considerable scraping. The epidermal cells were unavoidably scraped off at the same time, but these furnished very little material, and that was for the most part removed by washing. In any case their presence constituted a source of error which was probably practically constant throughout the series. The hair, after a thorough washing in water, was treated for ten to twenty minutes with dilute HCl (5 per cent) in order to remove any calcium which might remain in association with it. After further repeated washings, it was dehydrated in 95 per cent alcohol, subjected to ether for one hour.

Acknowledgment is due to Dr. C. L. Alsberg, of the Bureau of Plant Industry, for valuable suggestions relating to certain steps in this process.

<sup>&</sup>lt;sup>10</sup> An equal number of skins of the two lots were always treated at the same time, so that constant differences in treatment were avoided.

to insure the removal of fats, and then dried in the air. Each lot (the total pelage derived from a single pelt) was now put into a weighing bottle and transferred to a vacuum desiccator, until the weight was found to have become constant (usually for about four days). The final weight was recorded to the ten-thousandth of a gram, though only thousandths have been regarded in the present paper. Since a number of specimens were dealt with at once, the process did not require as much time as might be inferred from the number of steps involved. The results obtained are presented below (pp. 129-131).

Other characters chosen for measurement were: weight, length of body (from snout to anus), length of tail (from anus to tip), length of left ear (from the lower extremity of the incisura intertragica11 to the tip of the pinna), and length of left hind foot (from heel to tip of longest toe). Certain additional determinations, which were made in special cases, will be referred to at the proper time. A uniform method of procedure was adopted in making each of these measurements. In order that my figures may be of service for comparison with those of other workers in this field, it seems desirable to detail these methods a little more fully. The weight was taken by means of a torsion balance sensitive to a few hundredths of a gram. The tail length was obtained in two ways, according to whether the animal was living or dead when measured. When measured living, the mouse was suspended by the tip of the tail, the forefeet being allowed to rest upon the table. A certain degree of stretching generally occurred, the amount of which was found to average somewhat over 2 mm. in the case of full-grown mice. Weight and tail length were the only characters which it was possible to determine accurately with living animals.12 In measuring the tail of the dead mouse, and likewise the body length, the freshly killed animal was laid with the ventral surface uppermost upon a wooden board. A pin was passed through the roof of the mouth, thus securing the snout to the board. The latter was then held upright and the body allowed to dangle

<sup>&</sup>lt;sup>11</sup> Or at least a point which would seem to be homologous with the part so named in the human ear.

<sup>12</sup> I have since found it practicable to measure the ear and foot, in the living mouse, by the use of ether (April, 1909.)

for a few seconds, after which a second pin was passed through the basal part of the tail, which was thus likewise fastened securely in place. The distance from the tip of the snout to the anus and that from the latter to the tip of the straightened tail (exclusive of terminal hairs) were found by means of a graduated sliding caliper provided with a vernier. When the ear length was taken the mouse was laid freely upon the right side. In measuring the length of the right hind foot, the animal's body was held in the left hand, the sole of its foot pressed lightly against the board, and a pin passed through the instep into the wood. The tarsal joint was bent at about right angles, and the heel allowed to rise above the surface of support as in life. The caliper spanned the distance from the tip of the heel, which was touched rather lightly, to the tip of the nail of the middle (third) toe. It was found important to make all of these measurements as soon after death as possible. In the later series, two determinations were made of each part measured, and the reading was taken in tenths of a millimeter.

Throughout the whole of this work, the practice was followed, so far as possible, of measuring warm-room and cold-room individuals alternately, or at least of alternating small groups of individuals. Only thus would such gradual changes in one's standards of judgment as would inevitably result from growing experience, affect equally the two groups to be compared.

The characters selected are few in number, we must allow, and are, for the most part, such as would not be expected to respond adaptively to the treatment accorded to the animals. One must, however, accept the limits imposed by brevity of time and the nature of the material at hand. When subjected to statistical treatment a very few characters are found to involve a very great amount of labor. Moreover, aside from thickness of fur, such adaptations as might be conceived of as resulting from temperature conditions would probably be histological or chemical in their nature and therefore not accessible to ready methods of quantitative treatment.

The measurements which I have chosen as being applicable to these mice are, with the exception of those relating to the hair,

ones which are employed by mammalologists who concern themselves with rodents. Coues and Allen, 18 for example, give the following external measurements for Muridæ, Leporidæ, Sciuridæ, etc.: From tip of nose to (1) eye, (2) ear, (3) occiput, (4) tail; length of tail to end of (1) vertebræ, (2) hairs; length of fore-foot, and hind foot "from the tuberosity of the heel to the end of the longest claw"; height of ear. Merriam,14 likewise, presents figures for "total length" (i. e., body plus tail); "tail vertebræ" (i. e., length of the vertebral portion of the tail); "pencil" (tuft of hair at tip of tail); "hind foot;" "ear from crown" (sometimes from "anterior root" or from "notch"); and occasionally some others, including weight. Various dental and skeletal features are of course included in the complete diagnosis of a species, as well as differences in the color, texture and length of the pelage. But many of these characters are such as do not lend themselves to measurement, while others require the preparation of cleaned skeletons.

## STATISTICAL METHODS

Since it is to be presumed that many biologists are still unfamiliar with the methods of biometry, the following statement as to those employed in the present paper may not be out of place.<sup>15</sup> For a really earnest and, on the whole, successful endeavor to render this difficult subject intelligible to the non-mathematical mind, the reader is referred to Thorndike's "Introduction to the Theory of Mental and Social Measurements" (Science Press, 1904). Davenport's "Statistical Methods" is of course indispensable to those who are already sufficiently familiar with the use of these methods.

The mean or average of a series of values (in the present case,

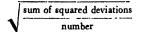
<sup>&</sup>lt;sup>13</sup> Monographs of North American Rodentia. Report of the U. S. Geological Survey, vol. xi, 1877, pp. 1-1091.

<sup>16 &</sup>quot;North American Fauna" series, published by the Biological Survey of the Department of Agriculture.

<sup>&</sup>lt;sup>18</sup>My thanks are due to Messrs. E. L. Thorndike and R. P. Bigelow for criticising certain portions of my manuscript and to Prof. C. B. Davenport for important information relative to biometrical methods.

measurements) is obtained by dividing the sum of all the terms of the series by the number of these terms.

The standard deviation, which is, at present, most frequently employed as the measure of the variability of a series, is obtained by squaring all the individual "deviations" or departures from the mean, finding the sum, and then the average, of these squared deviations, and extracting the square root of the resulting average, i. e.,



The reliability of the average or mean has, in each case, been indicated by the probable error, which is the number preceded by the  $\pm$  sign and annexed to the mean in the following tables. The value of this number is such that there is an even chance that the true mean (i. e., such as would be obtained from averaging an infinite number of terms) lies within the limits indicated by: given mean  $\pm$  probable error. The probable error of an average or mean is obtained by the formula

The reliability of an average thus varies inversely as the variability of the series and directly as the square root of the number of terms. The probable error of the mean, here employed as an index of reliability, is not to be confused with the probable error of a series, sometimes employed as an index of its variability. This latter is a number of such magnitude that it is exceeded by exactly one-half of the deviations. It has the value: .6745 × standard deviation.

The reliability of the standard deviation, or figure denoting the variability of each series, is indicated by the probable error of the standard deviation. This is obtained by the formula:

Since one of the primary objects of such investigations as the present is an inquiry into the effects of differences in the conditions of life upon supposedly homogeneous material, one of the principal points to be determined is the significance of any differences which may be discovered between the average values of a given character in two groups of individuals whose history has differed. For this purpose it is necessary to compare the probable error of the difference with the actually obtained difference between the two averages in question. The probable error of the difference is expressed by the formula:

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√ (probable error of the first average)<sup>2</sup> + (probable error of the second average)<sup>2</sup>
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i. e, the square root of the sum of the squares of the probable errors of the two respective averages. Now the actually obtained difference is the most probably true difference and it is as likely to be too small as too large. Nevertheless the true difference may possibly equal 0, i. e., be non-existent, in which case the obtained difference would be regarded as wholly "accidental." From the table of the values of the "probability integral" it may be calculated that the chances that a difference between two averages is due to mere accident are:

```
out of 1000 when difference between averages = 1 × probable error of the difference.

89 out of 1000 when difference between averages = 2 × probable error of the difference.

46 out of 1000 when difference between averages = 2 × probable error of the difference.

46 out of 1000 when difference between averages = 2.5 × probable error of the difference.

47 out of 1000 when difference between averages = 3 × probable error of the difference.

48 out of 1000 when difference between averages = 3.5 × probable error of the difference.

49 out of 1000 when difference between averages = 4.5 × probable error of the difference.

40 out of 1000 when difference between averages = 4.5 × probable error of the difference.
```

In proportion as the probability decreases that such a difference has been due to mere chance or accident (i. e., that it is the result of a multitude of independent causes having no relation to the conditions of the experiment), it is obvious that the probability increases that some constant modifying influence has been operative in differentiating the two groups. It must be admitted however, that the probabilities here stated apply in full strictness

only to cases where we are dealing with large numbers of indi-Davenport suggests 200 as the minimum number of individuals to be gathered for statistical treatment when the material is available; though he grants that much smaller numbers may be employed to advantage, where we are restricted by circumstances. But it must be borne in mind, he says16 "that the rules for determination of averages, probable errors, standard deviations and all the rest become less and less significant as the number of variants becomes smaller. Finally, in the region of twenty or so, the results can no longer be treated by mass statistics; twenty hardly makes a mass." To the experimentalist it must often happen, as in the present work, that the use of a large number of individuals, in any single series, is excluded by reason of the laboriousness of the methods employed. In such cases, we are told, no exact mathematical equivalent can be offered for the probability of a given result, even though the frequency distributions afford strong evidence on the subject. Of course the cumulative testimony of several independent series of experiments is of high value. In general, it would seem that the experimentalist demands a somewhat different statistical technique from the student of variation per se, and it is to be regarded as unfortunate that the methods at our disposal, have, thus far been developed mainly by the latter type of investigator, and with very little reference to the special needs of the former. To the experimentalist, who is studying the effects of artificial conditions, it is the significance of differences, and scarcely anythingelse in the whole field of statistical theory, that is likely to be of interest.

Regarding the accuracy of my computations, I can only say that every step has been gone over at least twice, and that, wherever possible, a different method of calculation has been employed in the repetition.

## RESULTS IN DETAIL Series of 1906-1907

Owing to the small number of individuals used and the tentative character of my methods at the outset, this first series will not be

<sup>16</sup> In a letter to the author

discussed at all fully. The mean temperatures in the two rooms, during the period of the experiment, were 24.9° C. and 9.1° C. (76.8° F. and 48.4° F.) respectively. No further analysis of the temperature conditions seems worth while in the present experiment. The humidity was not at any time determined. Twentyone mice (13 males and 8 females) were reared in the cold room; 20 mice (12 males and 8 females) in the warm room. The animals were not subjected to the differing temperatures until they were about three weeks old (21 ± days). Previous to that time, the undivided lots had been reared under similar conditions. Each lot comprised individuals from 7 different broods, each of the latter having been divided into two portions destined for the warm and cold rooms respectively. About half the stock, consisting of the broods earliest obtained, were subjected to the experimental conditions for a period of 106 days, the remainder for a period of 83 days. At the expiration of these terms the mice were paired for breeding purposes, and the two contrasted lots were transferred to a single room having a temperature somewhat intermediate between those previously employed. None of the animals were killed immediately after this transfer, while the females were kept until they had reared their broods. The interval between the discontinuance of the temperature differences and the killing of the animals varied from 15 to 55 days. Thus the material was far from homogeneous. Nevertheless the figures obtained seem worth recording. (Table 1.)17

The difference in tail length, in the males, at least, was often obvious without measurement, and it must be regarded as a significant one, even without such overwhelming corroborative evidence as is offered later. In the case of the females, the difference is much smaller, though it is greatly in excess of the probable errors. No further analysis of this table seems called for.

In the present series, the number of hairs per unit of skin area was computed for each mouse, according to the method described

<sup>&</sup>lt;sup>7</sup> Here and elsewhere the number of individuals measured for any given character is indicated by the figure in parentheses at the head of each column. In some cases individual tail measurements have been thrown out, where the tip of the organ had obviously been lost through accident. I trust, however, that it is not necessary to urge that no merely "exceptional" figures have been rejected

TABLE 1 Series of 1006–1007

		WEIGHT IN GRAMS	N GRAMS			BODY LENGTH	ENGTH			TAIL LENTGH	итсн	
	Maks	<b></b>	Females	les	Malcs	8	Females	iles	Males	8	Females	8
	Warm (12)	Cold (13)	Warm (8)	Cold (8)	Warm (12)	Cold (12)	Warm (8)	Cold (8)	Warm (10)	Cold (12)	Cold Warm (12) (6)	Cold (8)
Mean	24.742	24.742 24.792	23.437 24.437	24.437	6 80.46	93.67	92.25	93.25	88.10		77.67 84.50	80.20
Standard deviation	1.170	1.170 1.456 2.232 2.531	2.232	2.531	1.75	1.75 1.70 2.28	2.28	2.86	3.21	3.04	2.29	4.02

TABLE 2
Series of 1906-1907: Number of hairs per unit of skin surface

	M	ALES	FI	EMALES
	Warm (12)	Cold (13)	Warm (8)	Cold (8)
	165 297	164 146 310	190 394	<sup>242</sup> <sub>220</sub> 462
	164 137 301	144 174 318	140 174 314	174 182 356
	138 135} 273	154 178 332	189 338	165 315
	113 250	159 350	164 162 326	136 159 295
	95 205	150 310	144 292	127 263
	157 280	143 \ 95 238	145 161 306	158 305
	128 97 225	161 196 357	160 377	152 291
	109 232	148 187 335	111 220	151 310
	159 322 4	192 310		
	137 130} 267	161 170 331	1	
	135 287	140) 113) 253		
	122 144} 266	159 135		
	•	114 230		
ean	267.08±6.12	305.23± 7.37	320.87 ± 11.96	324.62 ± 13.68
andard deviation	31.37±4.32	39.43± 5.22	50.12± 8.45	57.40± 9.67

above (p. 101). It seems worth while to present the individual figures obtained for these animals, since this is the only series with which the counting method was employed. In Table 2 each figure in the left-hand columns represents the number of hairs on a circular disk of skin 1.5 mm. in diameter. Two figures are given for each individual, based upon two disks of skin taken at a distance of 1 cm. to the right and the left respectively of the middorsal line. The sum of these two is stated in heavy type.

It will be noted that the mean number of hairs for the cold room males is 305.23 upon the two disks, that for the warm-room males being 267.08.18 Here, then, is a difference of 14.3 per cent. The number of individuals is small, it is true, and the probable errors are large. Even granting the constancy, however, of such a difference, between two lots of mice thus treated, it is not necessary to conclude that there has been an actual increase in the number of (developed) hairs per unit of skin surface. If the warm-room individuals be supposed to have slightly thinner skins than those of the cold-room lot, the greater degree of stretching in the former (see p. 101) would result in a less dense distribution of hair upon its surface. But whether a difference so produced would be as high as 14 per cent may well be questioned. Again, it must not be supposed that I am urging this difference in the density of the coat of hair as an instance of permanent morphological change. It may be due merely to a difference in the rate at which the hair is shed. This point will be discussed later.

The averages for the hairs of the female mice are not far from equal in the two contrasted lots. It has already been noted that the females exhibited a much smaller difference in tail length than did the males.

## Series of 1907-1908

During the winter of 1907-1908 the experiments were conducted on a much larger scale than previously, the conditions employed were such as were calculated to result in the production of greater

<sup>18</sup> The number of hairs per square millimeter of skin may be readily computed, since the area of each disk was (approximately) 1.767 sq. mm. Thus the mean number for the cold room males is 86.4, that for all the mice comprised in the table is 85.0, etc.

modifications, and the number of measurements applied to the animals was considerably increased.

Temperature. The mean temperature<sup>10</sup> of the warm room for the entire season was 26.30° C. (79.34° F.), that for the cold room 6.16° C. (43.08° F.). These figures correspond roughly to those for the mean annual temperatures of Key West or Porto Rico, on the one hand, and those for Eastport, Maine, or Minneapolis, Minn., on the other.<sup>20</sup> But it would not, of course, be at all fair to compare the temperature conditions of the experimental rooms with those of the points named, still less to compare the climatic conditions as a whole.

The mean daily range of temperature (i. e., mean difference between the maximum and minimum for each day) was 11.9° C. (21.4° F.) in the warm room, 6.7° C. (12° F.) in the cold room. The maximum temperature reached at any time in the warm room was about 40° C. (for very brief periods), the minimum in the cold room being – 14.4°. But these figures represent exceptional occurrences and have little significance. Curves have been constructed (Fig. 1) showing the mean daily temperature in each room during the entire period of the experiment.

It is plain, of course, that none of these figures can represent the actual temperatures to which the mice themselves were most of the time exposed. When huddled together in large numbers in a nest of cotton-waste, the temperature of the air immediately in contact with them, at least in the case of the cold-room animals, was doubtless very much higher than that in the room outside, i. e., that recorded by the thermograph. Nevertheless, we all know by experience the difference between sleeping in a cold room and sleeping in a warm one, even when the amount of bedding is varied to suit the circumstances. And it must be remembered that during part of the time the mice were feeding, exploring the cages, etc., and were then wholly exposed to the air.

Humidity. The relative humidity in the warm room (see p. 100 above) ranged from about 22 per cent to about 66 per cent, the

<sup>&</sup>lt;sup>10</sup> The mean temperatures here given are based upon four figures daily, these being taken from the thermograph sheets.

<sup>20</sup> Report U. S. Weather Bureau for 1906-1907.

mean for 12 determinations made during a period of four months being 39 per cent. This is somewhat less than the mean relative humidity at Phoenix, Arizona, for the year 1906, as stated by the U. S. Weather Bureau, that point having the dryest atmosphere, with a single exception, of any place in the United States for which records are given. In general, the humidity of the warm room varied inversely as the temperature, since no compensation was made by the evaporation of water; but the degree of saturation of the outside air must have been a factor. The humidity of the cold room varied from about 49 per cent to about 90 per cent, the mean of nine determinations being 67 per cent. This figure is a trifle less than that given for the mean humidity of Philadelphia during the year 1906.

The physiological results of such differences in humidity must be far reaching. The quality of the air inspired must affect the processes of respiration, and the rate of evaporation from the body surface must differ widely in the two cases. This last was shown by the eagerness for water displayed by the warm-room individuals. To what degree the results which I offer have been brought about by differences in temperature and to what degree by differences in humidity it is impossible to state. In these experiments, it has not been practicable to control the humidity, independently of the temperature, and thus it has been impossible to decide this question definitely. The subject will be referred to later.

Disposition of Stock. Twenty broods of young mice, aggregating 135 individuals, were employed for this experiment. In order to insure, so far as possible, a division into two lots of a similar hereditary endowment, one-half the individuals of each litter were subjected to the high temperature, the other half to the low. For this purpose, exchanges were made between the offspring of different mothers according to the following plan: One-half of brood A, plus one-half of brood B, were consigned to the care of the A mother in the cold room, the other half of each brood being consigned to the care of the B mother in the warm room, and so on through the series. This disposition of the young was made while the latter were 2 to 4 days old (in a few exceptional cases as much as 11 days). The members of each litter were marked at the

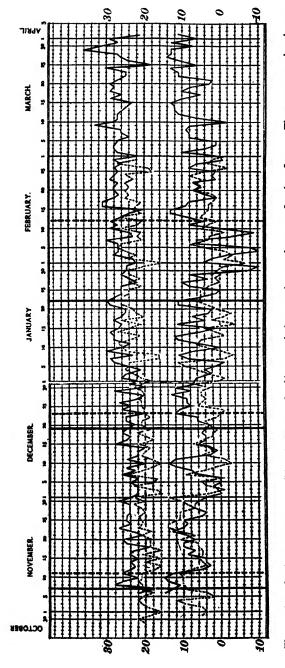


Fig. 1 Chart showing temperature conditions in warm and cold rooms during experiments of 1907-1908 and 1908-1909. These curves are based upon the mean temperatures for each day, as computed from four daily readings at equal intervals. The continuous lines represent the temperatures for the earlier winter's experiments, the broken lines those for the later year. The heavy vertical lines (continuous and broken) denote (1) mean date of birth of each year's mice; (2) mean date at which the 42-day measurements were made in each year; (3) mean date at which the 2\$-month and 3-month measurements were made.

outset by a system of clipping the right ear and various fingers and toes of the right foot. Rather contrary to my expectations, the alien offspring were accepted by the mothers as readily as their own, so that little if any loss of life resulted from this procedure.

A certain proportion of deaths occurred here as always during the rearing of these mice. The number of deaths in the warm room during the first six weeks was 6, giving a mortality of about 9 per cent. The number dying in the cold room was considerably greater, being 13, or about 20 per cent of the lot. During the next month of life no deaths occurred among the warm room lot, while 6 were recorded in the cold room; but scarcely any further deaths from natural causes occurred during the next four months, i. e., until the end of the experiment. The cold room individuals, throughout the earlier part of their life, at least, were much less active than those in the warm room. During the first few weeks they kept to their nests almost constantly. Nevertheless, when mature, they were of decidedly better appearance than the warm room lot, and, when paired, they reared a much higher percentage of offspring. It must be added, however, that the reproductive capacity of both lots was found to be distressingly slight—so slight, in fact, as to render futile any attempt to make a satisfactory test of the transmissibility of the modifications which had been produced. Among the 21 females in the cold room lot, 31 pregnancies are recorded for the 15 weeks during which they were kept with the males, while in the aggregate only 48 young were reared to the age of six weeks. Indeed, the majority of the litters either consisted entirely of stillborn young, or of ones which died during the first few days after birth. In other cases, the young were apparently born healthy, but the mothers seemed unable to suckle them or perhaps lacked the instinct to do so. With the warm room lot the case was even worse. Of the young resulting from 50 pregnancies (doubtless over 200) only 35 individuals, or about 15 per cent, survived to the age of six weeks, while in the great majority of litters all the individuals died either before or shortly after birth. I am still almost wholly at a loss to account for this failure of the powers of reproduction. The mice were paired rather too young, it is true, being 2½ months old at the time. Many of them did not become pregnant till they were much older than this, however, while it is well known that female mice may bear healthy young at an even earlier age. Again, when judged by most other standards, the animals appeared to be in perfect health. They were active enough, and the fur was commonly in good condition, though the size of the females, at least, even when fully grown, was probably somewhat below normal. Moreover, after the earlier weeks of life, their mortality had been slight. That this damage to the generative powers must be set down as one of the results of the experimental conditions seems, nevertheless, probable.

Measurements at 42 Days. The weight and tail length of these mice was taken at the age of six weeks.<sup>21</sup> No other measurements were at that time believed to be practicable with the living animals. The following table (no. 3) presents the mean and the index of variability for each of these measurements, the two sexes being treated separately.

TABLE 3
Series of 1907-1908: Measurements at six weeks of age

İ		WEI	GHT		}	TAIL L	ENGTH	
	Ma	ıles	Fem	ales	M	ales	Fem	ales
	Warm (29)	Cold (31)	Warm (33)	Cold (23)	Warm (29)	Cold (31)	Warm (33)	Cold (23)
Mean		±0.374	±0.179	±0.301	±0.35	±0.68	±0.43	±0.83
		±0.265				, ,	, ,	, ,

Fig. 2 shows the distribution of weights for the cold and warm room groups (sexes combined); Fig. 3 shows the distribution of tail lengths for the two groups. From the table and polygons collectively the following facts may be gathered:

Nowing to difference in the date of birth, this age was not attained simultaneously. The great majority, however, were born within the space of a week.

The tails of the warm room individuals are much longer than those of the cold room individuals. This difference amounts to 27.1 per cent for the males, 33.0 per cent for the females, and 29.7 per cent for the sexes combined. Indeed, the two types are so distinct that, but for a single individual, there would be no overlapping of the polygons; i. e. (barring this single exception), the longest tail in the cold room lot was shorter than the shortest in the warm room lot. These differences were so patent to the eye that, had the two lots of mice been mixed together accidentally, I am

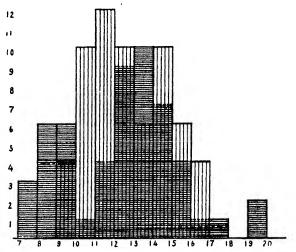


Fig. 2 Series of 1907-1908: weight at six weeks of age (sexes combined). Abscissas denote weight in grams; ordinates denote number of individuals. Vertically shaded areas represent warm-room animals; horizontally shaded areas represent cold-room animals.

sure that I should have been able to separate them again with comparatively few mistakes. Contrary to the condition in the first year's series, a greater difference is here shown by the females.

2 The warm room males were on the average 1 per cent lighter than the cold room males; the warm room females, on the contrary, were 7.7 per cent heavier. Quite similar relations in respect to weight will be found in the series of the following year. The frequency polygons for weight show that two groups of individuals, having two different "modes," are comprised in the cold room lot—a lighter and a heavier group. An analysis of the individual

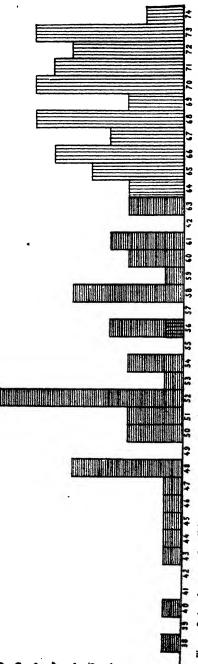


Fig. 3 Series of 1907-1908: tail length at six weeks of age (sexes combined). Abscissas denote tail length in millimeters; ordinates denote number of individuals. Shading as before.

figures shows that both the males and the females are divided quite sharply into these two groups. The number of animals is so small, however, that this phenomenon may be accidental. It will be referred to later.

- 3 In the case of both sexes, the variability both in weight and in tail length is considerably greater for the cold room lot than for the warm room lot.
- 4 The mean weight of the males (irrespective of history) is considerably greater (9.6 per cent) than that of the females; the tail length seems to bear no constant relation to sex, nor does the variability of either character.

Measurements at 2½ Months. At the age of 2½ months the mice were mated. At about the same time (when 75 to 78 days old) the measurements previously made (weight and tail length) were repeated. The number of females surviving to this age was 54 (33 warm + 21 cold). The number of males reserved for breeding was 13 (7 warm + 6 cold). There remained 43 males (22 warm + 21 cold) which were not needed for breeding purposes. They were therefore killed at this time, and subjected to a considerably greater number of measurements than had hitherto been employed. At this period, therefore, there are to be considered three groups of individuals (each group consisting, of course, of a warm room and a cold room half): (1) the females; (2) the mated males; (3) the unmated males.

Referring to Table 4, it will be seen that among the female mice the warm room lot are 4.2 per cent heavier than the cold room lot, while the tail length of the former is 26.6 per cent greater. Comparing the mean figures here given with those of the table for 42 days, it will be found that in the cold room lot the mean weight has undergone an increase of 33.1 per cent during the interval between the two measurements, while the mean tail length has undergone an increase of 16 per cent.<sup>22</sup> In the warm room lot,

<sup>&</sup>lt;sup>28</sup> Of course this increase in the mean has not exactly the same value as the average individual increase. The latter figure cannot be given for the present series, since the mice had not been marked so as to be individually distinguishable, although the members of each litter were identified by a brood number. Inasmuch as there had been but two deaths in the interval between the measurements, we are dealing with practically the same group in each case.

on the other hand, the increase in weight and in tail length have been 28.8 per cent and 10.3 per cent respectively. There has thus been manifested a tendency toward equalization in respect to both of these characters, but especially in respect to tail length. This appendage has undergone a percentage increase which has been more than half again as rapid in the case of the cold room (i. e., the shorter tailed) as in the warm room (i. e., longer tailed) animals. Further evidence for such a general tendency toward the neutralization of early differences will be offered later. As regards variability, the standard deviation for tail length, both in the warm and cold room lots, has undergone a slight absolute decrease,

TABLE 4
Series of 1907-1908: Females 24 months old

	WEI	GHT	TAIL LEI	NGTH
	Warm (33)	Cold (21)	Warm (33)	Cold (21)
Mean	15.821 ± 0.261	15.176± 0.330	76.18 ± 0.38 ;	60.19 ± 0.76
Standard deviation	2.221 ± 0.184	2.236± 0.233	3.20 ± 0.27	5.10 ± 0.53

notwithstanding a considerable *increase* in the average for each lot. The standard deviation for weight, on the contrary, has undergone an increase in each lot. The relative variability (i. e., ratio of standard deviation to mean) has increased in one case (warm room), decreased in the other (cold room).

TABLE 5
Series of 1907-1908: Mated males 2½ months old

particular designation to the state of the state of			7	
i 1	WE	IGHT	TAIL I	ENGTH
	Warm (7)	Cold (6)	Warm (7)	Cold (6)
Mean	17.843 ± 0.568	20.117± 0.824	78.∞ ± 1.17	66.00 ± 0.62

No discussion of the "mated males" (Table 5)<sup>28</sup> is worth while, owing to the small number of individuals comprised. These

<sup>&</sup>lt;sup>26</sup> A certain degree of selection was exercised in picking out these males, the larger individuals of a brood being chosen for breeding purposes. This is shown by the difference in mean weight between the "mated males" and the "unmated males."

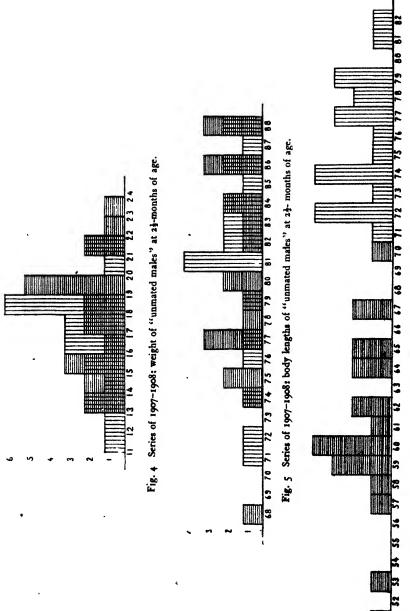


Fig. 6 Series of 1907-1908: tail length of "unmated males" at 24 months of age.

TABLE 6 A

Series of 1907-1908: Males (unmated) killed at 2½ months.

WARM ROOM (22) Caudal Hair in Body Weight Tail Foot verte-Ear length mg. brae 82 16.9 328 15.5 74 32 13.2 14.3 76 72 12.6 17.1 146 33 15.1 82 13.6 79 32, 17.3 350 13.8 18.2 198 72 72 12.7 33 18.8 83 13.0 17.0 74 32 343 17.7 81 78 17.2 33 12.4 341 13.0 16.4 228 77 12.5 73 34 18.3 86 13.0 17.0 271 77 32 12.3 71 31 13.0 17.3 198 74 16.6 83 289 77 32 12.3 17.5 21.6 88 79 32 13.2 17.5 371 21.7 84 16.9 318 79 31 13.5 81 17.9 17.3 74 31 13.0 233 16.8 81 72 12.3 17.2 258 33 18.3 87 13.6 17.6 279 77 33 18.7 85 12.9 17.2 252 72 31 16.1 81 76 32 13.2 17.6 229 8.11 74 71 32 13.3 17.5 130 18.8 84 18.1 261 79 32 13.5 17.2 82 78 32 13.2 17.4 234 18.7 86 81 258 32 13.1 17.5 20.8 88 31 13.0 17.6 306 75 Mean.... 16.964 81.41 264.59 13.005 75.73 32.09 17.359 ±0.346 ±0.69 ±0.056 士8.98 ±0.45 土0.11 ±0.055 Standard 0.389 0.382 4.78 62.48 2.724 3.11 0.74 deviation... ±0.277 ±0.08 ±0.49 ±0.32 士6.35

TABLE 6B .
Series of 1907–1908: (Males unmated) killed in 2½ months.

Series of 1907-1908: (Males unmated) killed in 24 months.

COLD ROOM (21)

	Weight	<b>B</b> ody length	Tail	Caudal vertebræ	Ear	Font	Hair in mg.
	21.4	83	59	31	12.6	16.4	462
	19.8	80	60	31	12.8	8.61	349
	14.9	74	59	31	12.3	16.5	237
	18.5	77	59	31	12.5	16.4	328
	17.6	79	60	32	12.6	17.1	330
	15.7	75	61	31	12.9	16.6	257
	15.2	80	57	[28]*	12.5	16.2	228
	13.2	75	62	32	12.2	17.4	233
	16.0	78	58	[29]*	12.0	16.0	205
	18.3	84	64	33	12.6	16.9	293
	15.5	77	60	32	12.3	16.7	239
	13.2	68	62	32	12.4	16.8	227
	23.1	88	64	33	13.5	17.5	364
	22.7	88	65	32	13.3	17.5	502
	14.7	77	51	30	12.2	16.3	211
	17.2	82	53	32	12.2	16.3	225
	21.8	86	60	31	13.0	16.9	284
	19.5	86	70	33	14.0	17.2	255
	19.3	88	67	30	13.4	17.3	284
	19.0	84	65	32	13.5	16.7	383
	19.4	86	67	31	13.7	17.3	294
Mean	17.905	80.71	61.10	31.58	12.786	16.800	294.76
	±0.425	土0.78	±0.65	±0.14	±0.082	±0.065	±11.66
Standard	2.888	5.33	4.41	0.88	0.558	0.441	79.17
deviation	±0.301	±0.55	±0.46	±0.09	±0.058	±0.046	±8.24

TABLE 6C

Series of 1907-1908: Males (unmated) killed at 23 months. Figures arranged for comparison

	-	-			-									
	WEIGHT	1.80	BODY 1	LENGTH	TAIL 1	LENGTH	, NUMBER VERTI	MUMBER CAUDAL VERTEBRAE	EV	EAR	٤	FOOT	WEIGHT OF HAIR IN MILLIGRAMS	OF HAIR IGRAMS
	Warm	Warm Cold	Warm	Warm Cold Warm	Warm	Cold	Warm	Cold	Warm	Cold	Warm	Cold	Warm	Cold
Mean	Mean 16.964 17.905 81.41 80.71 ±0.346 ±0.425 ±0.69 ±0.78	17.905 ±0.425	81.41 ±0.69	80.71 ±0.78	75.73 ±0.45	61.10 ±0.65	32.09 ±0.11	31.58	13.005		12.786 17.359 ±0.082 ±0.055	16.800 ±0.065	264.59 ±8.98	294.76 ±11.66
Standard deviation	2.724 ±0.277	1,888 ± ± 0.301	4.78 ±0.49	5.33 ±0.55	3.11	4.41 ±0.46	.7. ±0.08	0.88 ±0.09	0.389 ±0.040	0.558 ±0.058	0.382	0.441 ±0.046	0.441 62.48 ±0.046 ±6.35	79.17 ±8.24

figures will be later combined, however, with certain of those given for the next group.

The unmated males, measured at the age of  $2\frac{1}{2}$  months, constitute the most important group in the second year's series. Table 6 (A, B, and C) presents the measurements for these 43 mice. These measurements were all made after killing.

Comparison of these figures with those given for the males at 42 days is of course only possible in respect to two characters—weight and tail length. In order to determine accurately what changes have occurred in these, however, we must first combine the figures for the present group with the preceding group of "mated males," since the two together comprise the entire collection of males which had been measured earlier in life. Table 7 accordingly represents the mean weight and tail length for all males at the age of 2½ months.

TABLE 7

Series of 1907-1908: All males at 2½ months of age

	WEI	GHT	TAIL LI	ENGTH
-	Warm (29)	Cold (27)	Warm (29)	Cold (27)
Mean	17.176	18.396	77·41	63.33
	±0.331	±0.396	±0·44	±0.56
Standard deviation	2.646	3.053	3·54	4.28
	±0.234	±0.281	±0.31	±0.39

Comparing these figures with those of the males at six weeks of age, we note that whereas the tails of the cold room lot have increased 16.9 per cent in the interval between the measurements, those of the warm room lot have increased only 12.5 per cent. There is thus seen to be a tendency to "catch up" on the part of the less developed organs, which has already been pointed out for

<sup>&</sup>lt;sup>24</sup> In combining these figures an allowance is first necessary. The tail length of the dead mice was, as stated above (p. 103), obtained by a different method from that practised upon the living ones. I have found that in living mice of this size the tail is stretched on the average about 1.5 mm. during the suspension. This amount has accordingly been added to the mean tail length of the unmated males before combining with that of the mated males. The resulting figure represents approximately the tail length which would have been obtained had all been measured alive.

the females and will be discussed later. It must be added, however, that in the present case the difference in weight between the two groups has increased rather than diminished. In respect to variability, two of the four standard deviations comprised in the table show an absolute decrease, one of the others indicates a slightly lessened variability, while in the fourth case there is an increase, both absolute and relative. Not much importance is to be attached to these latter comparisons, however.

Returning to a consideration of the figures presented in Table 6 it is seen that the weight is 5.3 per cent less in the warm room lot than in the cold room lot; while the body is seven-tenths of one per cent longer and the tail 23.9 per cent longer. Passing to the new measurements (not applicable to living animals), the average length of the (left) ear is 1.7 per cent greater in the warm room lot; that of the foot 3.3 per cent greater. The average weight of hair (see p. 102) for the cold room mice is 11.4 per cent greater than that of the warm room individuals. It has seemed worth while to represent graphically the frequency distributions of these characters (Figs. 4 to 9). The difference in weight between the two sets of mice (Fig. 4) cannot with any certainty be regarded as a significant one. The difference in body length (Fig. 5) is too trivial to be taken into consideration.

In striking contrast, however, is the case for tail length (Fig. 6). Here there is no overlapping whatever of the polygons, while the modes are removed by a distance of 13 mm. Two questions present themselves respecting this difference of tail length: (1) Does it involve an actual difference in the volume of the organ? And (2) does it involve a difference in the number of vertebræ? In order to test the first question the diameter of the tail at its widest point was obtained by means of calipers for all the mice of this group. While this is a difficult measurement to make with any great accuracy, the figures are probably exact enough for present purposes. The averages for warm room and cold room animals are 2.94 mm. and 3.02 mm., respectively. There is thus the possibility of a slightly greater attenuation of the tail, accompanying its more rapid increase in length, but the former is certainly nothing like proportionate to the latter. There is

therefore an actual difference in the volume of the tail. The number of caudal vertebræ was likewise counted for all of this

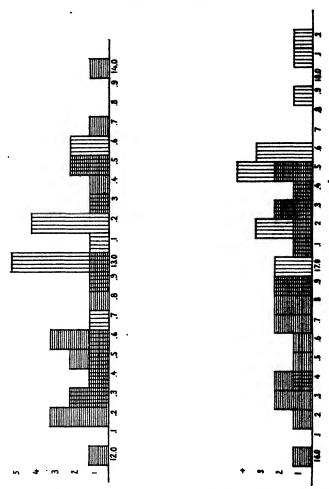


Fig. 7 Series of 1907-1908: ear length of "unmated males" at 2½ months of age.

Fig. 8 Series of 1907-1908: foot length of "unmated males" at 2½ months of age.

group. The mean figures are given in the table26 (6), where it is seen that the difference between the two groups (if significant

<sup>&</sup>lt;sup>26</sup> The number of vertebræ was counted, from the sacrum to the last nodule of bone distinguishable under a low power objective. The task was comparatively simple and admitted of tolerable accuracy. The average error of observation was probably less than one vertebra per mouse. Since the skele-

at all) amounts to only about half a vertebra, i. e., it is not sufficient to make any appreciable difference in the tail length. In fact there is very little deviation from the mean throughout the entire series. It would be surprising indeed if the number of vertebræ had been found to be altered by temperature conditions, first because this number does into in all probability vary very widely in most species of mammals, and, secondly, because the definitive division of the embryonic tissues into vertebræ is probably complete long before birth.

The present difference in ear length is not convincing, but foot length, has, with practical certainty, been affected by the tempeature conditions.

It must be remembered that we are here dealing with mice which differ among themselves widely in size, and that neither the mean weight nor the mean body length is quite the same for the two groups. In Table 8 are presented the *relative* magnitudes of certain characters. Here we have the mean ratios between the length of tail, ear and foot in each individual and the length of the body; likewise the ratios between the weight of the hair and the square of the body length. In the case of the ear and the foot, the variability of the ratios is found to be much greater than that of the absolute measurements. This is due to the fact that these parts vary but slightly as compared with the size of the animals. Indeed their length is remarkably constant throughout each series, irrespective of the body length of the individual.

The case of the hair deserves a rather full discussion, since this is the character, in particular, whose modification may be supposed to be of an adaptive nature. It is seen from Table 6 that the mean weight of the pelage for the cold room individuals is 11.4 percent greater than that of the warm room individuals. The variability is very high, to be sure, partly because the animals vary much in size, partly because they actually vary in the density of their hair

ton was not thoroughly denuded of muscles, etc., (alcoholic specimens were used) it was not always easy to distinguish the termination of the sacrum and the commencement of the caudal series, and an error of one or two vertebræ perhaps resulted occasionally from this cause. In a few instances, some of the minute terminal vertebræ were lacking, owing to obvious injury to the tail. In such cases, the figures have been enclosed in brackets and have not been included in making up the averages.

TABLE 8

	Series	veries of 1907-1908: Unmated males—ratios, expressed in percentages of body length	Unmated males	raios, expressed		of word tengen		
٠,	TAIL LENGTH:	TAIL LENGTH: BODY LENGTH	EAR LENGTH:	BODY LENGTH   FOOT LENGTH; BODY LENGTH	FOOT LENGTH:	BODY LENGTH	WEIGHT OF H.	WEIGHT OF HAIR IN GRANS: (BODY LENGTH) <sup>2</sup>
	Warm (22)	Warm (22) Cold (21)	Warm (11)	Cold (21)	Warm (22)	Cold (21)	Warm (22)	Cold (21)
Mean	93.23 ±0.71	75.86 ±0.83	16.023 ±0.141	15.871	21.391 ±0.206	20.895 ±0.191	0.03959	0.04501
Standard deviation	(, +.95 ±0.50	5.67 ±0.59	0.978 ±0.099	0.778 ±0.081	1 435 ±0.146	1.297	0.00771 ±0.00078	0.00975

coat. I have therefore made an endeavor to compute the relative amount of hair, making allowance for the area of the skin—that is, I have obtained the ratio between the hair weight of each mouse and the square of its body length. The mean of these figures is 0.04501 mg. per sq. mm. for the cold room lot, 0.03959 for the warm room lot. According to this computation, the cold room mice have a relative amount of hair 13.6 per cent greater (heavier) than the warm room ones. It must be admitted, however, that such a method of computation is open to criticism. To estimate the relative skin areas of these mice by comparing the squares of their body lengths presupposes that they are, in the language of geom-

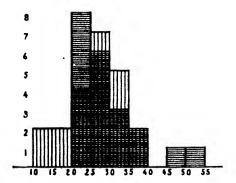


Fig. 9 Series of 1907-1908: weight of hair (absolute) of "unmated males" at 2½ months (expressed in hundredths of a gram).

etry, "similar solids," which they are not. As a matter of fact, while the warm room mice have a slightly greater bodylength than the contrasted group, they are lighter, on the average, by nearly one gram, i. e., a difference of over 5 per cent. They are probably somewhat less plump, therefore. I must add, however, that I do not believe any such slight difference of shape to be accountable for the difference in the amount of hair which is shown by the two groups of mice. The most serious criticism of these figures relates to the number of individuals, which is confessedly too small to permit of our drawing any final conclusions in the presence of such high variability.

Until further data are available, however, it seems worth while to offer them for what they are worth.

Fig. 9 shows the distribution of hair weight for the entire lot, both the hot room and the cold room individuals. No fair comparison can be drawn from these polygons, as has already been stated, since mice of very different sizes are represented. In Fig. 10 I have substituted in each case for the absolute weight the ratio of the hair weight to the square of the body length. While the modes lie at the same point, the centers of gravity are considerably separated.

In view of statements cited by Lydekker regarding certain modifications which are alleged to have been produced in cats by

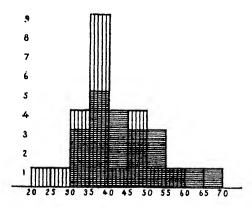


Fig. 10 Series of 1907-1908: weight of hair (relative) of "unmated males" at 2½ months (= ratio of hair-weight to square of body length).

life in a cold-storage warehouse, I endeavored to determine for the present group of mice whether there was any appreciable difference in the length of the vibrissæ between cold room and warm room individuals. The measurements have necessarily been rough in the extreme. The longest hair among the "whiskers" of one side was measured by calipers, without straightening or removing it. The mean figures obtained were 26.8 mm. for the warm room lot; 25.4 for the cold room lot. Considering the crudity of the method, the individual measurements vary compara-

<sup>26</sup> A Handbook to the Carnivora. Part I: Cats, etc.; pp. 158-163.

tively little, though enough, probably, to deprive this difference of any significance. No such obvious modifications as has been alleged for cats <sup>27</sup> is here evident.

Measurements at 7 Months. The subsequent history of the female mice varied considerably with different individuals, according to the exigencies of the breeding experiments. Each female from either lot, as she became pregnant, was transferred to a room kept at a temperature somewhat intermediate between the hot and cold rooms. If, as was commonly the case (see p. 116), her brood did not survive, she was taken back to the room from which she came. Thus, in the interval between February 6 and April I, a considerable proportion of the females were transferred back and forth between the warm or cold room and the "intermediate" room, in some cases more than once. I have not thought it worth while to compute the average duration of each set of conditions for the lot. On April 1, all the mice were moved to the "intermediate" room and the sexes separated. On May 1, they were paired again, but with the same unfortunate results. The entire lot of females was killed and measured between June 4 and July 6. All were about 7 months of age at the time of killing, save for a few mothers of broods, which were allowed to remain with the latter till they were old enough to take care of themselves. These somewhat older individuals (7½ to 8 months) have, however, been included in the table herewith given. As they were, with little doubt, all fully grown, this proceedure seems fair.

The mean figures obtained for each lot of females is given in Table 9. It herewith appears that the weight in the warm room lot is 2.4 per cent greater than in the cold room lot, the body two-tenths per cent longer, the tail 14.9 per cent longer, while the average ear length is practically equal in the

<sup>&</sup>lt;sup>27</sup> It is true that in the case cited by Lydekker the elongation of the vibrissæ was attributed to the darkness, rather than to the cold.

<sup>&</sup>lt;sup>28</sup> It seems probable that, so far as the tail at least is concerned, the cold-room mice have departed from the more usual or normal condition, while the warm-room individuals have been little if any modified. Fifty-nine adult female mice, of unknown history, which were received by me during the present winter, had a mean tail length of 92.8 mm.; i. e., their tails were considerably longer than even the warm-room females of Table 7. It must be noted, however, that they were larger mice, having a mean weight of 26.6 gms.

TABLE 9 Series of 1907-1908: Females about 7 months old

						-	-			
	WE	WEIGHT	BODY	BODY LENGTH	TAIL L	TAIL LENGTH (LIVING)	M	EAR	<b>i</b> .	FOOT
	Warm (27)	Cold (18)	Warm (27)	Cold (18)	Warm (27)	Cold (18)	Warm (27)	Cold (18)	Warm (27)	Cold (18)
Mean	±3.059	22.522 ±0.514	94.03 ±0.56	93.84 ±0.58	86.96 ±0.38	75.67 ±0.78	13.743 ±0.048	13.703	17.322 ±0.050	16.631 ±0.091
Standard deviation:	3.457	3.233 ±0.363	4·35 ±0.40	3.62	2.93	+.90 ±0.55	. 0.368 . ±0.034	4.90 : 0.368   0.464 ±0.55 : ±0.034 : ±0.052	0.382 ±0.035	0.573 ±0.064

TABLE 10 Series of 1907-1908: Males "mated," about 7 months old

	WEIGHT	THE	BODT LENGTH	ENGTH	TAIL LENGTH (LIVING)	(пинд)	LAR		FOOT	н
	Warm (7)	P 9	Warm (7)	Cold (6)	Warm (7)	Cold (6)	Warm :	Cold (6)	Warm (7)	Cold (6)
Mean .	23.657 2 1 ±0.375 ±	25.167 ±0.440	홌뷳	11 95.69 18 86 ±0.58 ±	87.00 ±0.\$\$	77.83 ±0.56	14.043 14.067	14.067	17.771	17.283

two lots. None of these differences except those in the length of the tail and foot are to be regarded as having any significance.

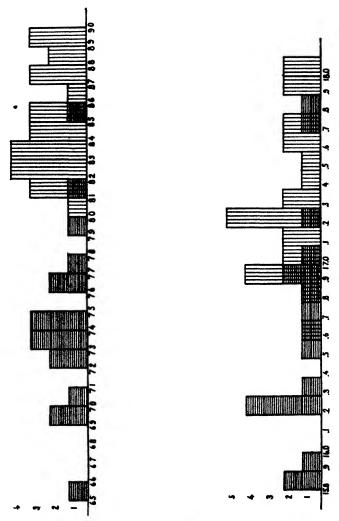


Fig. 11. Series of 1907-1908; tail length of females at 7 months of age (based upon measurements after death—see text.)

Fig. 12 Series of 1907-1908: foot length of emales at 7 months of age.

Comparing the measurements for this age with those (weight and tail) made upon these same mice<sup>20</sup> when 2½ months old, we find

<sup>&</sup>quot;Six of the warm-room lot and three of the cold-room lot had died in the meantime.

that the weight has increased during this interval 48.4 per cent in the cold room (i. e., lighter) lot; 45.8 per cent in the warm room (heavier) lot. The gain in tail length for the cold room lot has been 25.7 per cent, as compared with an increase of only 14.2 per cent on the part of the warm room individuals. In respect to each character, therefore, but more especially in respect to the tail, there has been a very obvious tendency toward a diminution of the differences between the two contrasted sets.

Referring to variability, a comparison of the standard deviations for tail length shows that there has been a slight reduction in the absolute and a considerable reduction in the relative variability in both the warm room and cold room lots. The standard deviations for weight have undergone an increase in each lot, though the relative variability has remained practically unchanged.

Frequency polygons (Figs. 11 and 12) have been plotted for tail length and foot length in this group of females. The polygons for the former overlap to a very slight extent; those for the latter are sufficiently distinct to admit of no doubt as to their significance.

The males used for breeding were kept under the extreme temperature conditions until April 1, at which time they were 4 to 5 months old. They were then transferred to the "intermediate room" along with the females, and were killed upon reaching the age of 7 months.

The measurements for this group are given in Table 10. Comparing the present figures with those for the same mice at the age of  $2\frac{1}{2}$  months, we find that the lighter warm room lot has gained to the extent of 32.7 per cent of its former weight, while the heavier cold room lot has gained only 25.1 per cent, again showing a tendency toward equalization. The same fact is evident in the growth of the tail. This has amounted to 17.9 per cent in the case of the warm room lot.

## Levelling Down of Early Differences.

Reference has more than once been made, in discussing particular sets of measurements, to a tendency for these experimentally produced differences to diminish with growth. A table has been

prepared (Table 11) which includes all the cases in which this point can be tested.

TABLE 11
Series of 1907-1908: percentages of increase\* in the intervals between successive measurements

	1	EEKS MEASURE- NTS	2½ MC PERCENT INCR	AGES OF	7 MON PERCENT	AGES OF
	Weight	Tail length	Weight	Tail length	Weight	Tail length
Males \{\begin{aligned} \text{Warm} \\ \text{Cold} \\  \end{aligned}	12.997	68 8 <sub>3</sub> 54.16	32.1% 40.2%	12.5% 16.9%	.32.7% 25.1%	11.5% 17.9%
Females \begin{cases} \text{Warm} \\ \text{Cold}	12.282	69.06 51.91	28.8% 33.1%	10.3% 16.0%	45.8% 48.4%	14.2% 25.7%

<sup>\*</sup>See foot note on p. 120 above.

†In considering the figures for 7 months, it must be recalled that only 13 males (7 warm + 6 cold) have been kept till this time. In figuring percentages of increase for these males, therefore, the later figures have been compared with those for this same group of "mated males," and not with those for the males in general. In the column for 2½ months, on the contrary, the figures for "all males" are given.

Under the "6 weeks" column, we have the absolute measurements of weight and tail length for males and females belonging to the warm room and cold room lots. In the column for 23 months is given for each group the percentage of increase of each of these characters, during the interval between the two measurements. In the "7 months" column are given the percentages of increase over the measurements for 21 months. For each sex are presented two horizontal rows of figures: those for the "warm" and the "cold" lots respectively. It is thus easy to compare the contrasted figures for any one character. In each pair of these contrasted figures, that one has been printed in heavy type which represents the rate of increase for the group which had previously shown a lower mean value for the character in question. This group should, according to the hypothesis, be expected to have undergone a more rapid rate of increase. As a matter of fact, it will be noted that in 7 out of the 8 pairs of contrasted figures, the

TABLE 12 Series of 1908-1909: Weight and tail length at six weeks

		WE	WEIGHT			TAIL 1	TAIL LENGTH	
•	W	Males	Females	iles	Males	les	Fea	Females
	Warm (55)	Warm (55) Cold (50)	1	Cold (38)	Warm (43) Cold (38) Warm (53) Cold (47)	Cold (47)	Warm (42) . Cold (35)	. Cold (35)
Mean	. { 12.604 ±0.283	13.180 ±0.241	12.663	11.889 ±0 195	67.19 ±0.55	60.11 ±0.51	68.95 ±0.48	59.49 ±0.53
Standard deviation	3.119	2.522 ±0.170	2.107 ±0.153	1.783	5.98 ±0.39	5.20 ±0.36	4.61 ±0.34	4.63 ±0.37

number in heavy type is the larger number.<sup>30</sup> This decrease in bodily differences originally brought about by differences of temperature has not been due to a withdrawal of the latter. Indeed, during the interval between the "6 weeks" measurements and the "2½ months" measurements the temperature differences in the two rooms have increased rather than diminished. (See Fig. 1.) Later, it is true, the temperature differences gradually diminished, and commencing with April 2 they were abolished altogether. At the latter date, however, the mice averaged nearly five months old, and their growth was probably not far from complete.

While the tendency toward a reduction of the original differences between the warm and the cold room groups is thus pretty clear, the evidence for a reduction of variability within each group is not so certain. An inspection of the tables shows us twelve cases in which we can compare a later standard deviation with an earlier one for the same character. In six of these cases the later standard deviation is smaller, i. e., the decrease in variability has been absolute as well as relative. In two cases there has been a relative decrease, though not an absolute one; while in two others, the relative variability has remained practically unchanged. In only two of the twelve cases has there been any appreciable increase in the relative variability. In view of the lack of uniformity in these results, however, and the commonly high probable errors, too much significance must not be attached to them. It is worth pointing out, however, that the variability for tail length has decreased absolutely as well as relatively in five out of six cases.

## Series of 1908-1909

At the date of writing, the experiments of the third winter have not been carried very far. A first and second series of measurements upon the living mice have, however, been made, and the results seem well worth comparing with those of the preceding

<sup>&</sup>lt;sup>30</sup> It is likewise true that in all of these seven cases there has been a greater absolute gain as well as a greater percentage increase. Rate of growth seems more fairly expressed, however, in terms of proportionate increase.

year. 31 The temperature conditions during the first four months are indicated in Fig. 1, in which a certain degree of comparison with those of the previous winter is made possible. The mean temperature of the warm room during the first three months of

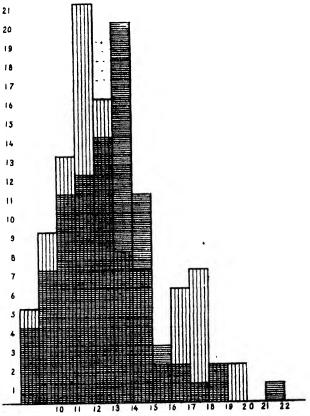


Fig. 13 Series of 1908-1909: weight at six weeks (sexes combined).

the animals' lives was 21.3° C., with a mean daily range of 12° C. In the cold room the mean temperature during this same period was 3.6° C., the range of daily fluctuation being 6°. The temper-

st The mice here used are from an entirely new stock, none of them being descendants of those raised during the preceding year. Animals from several different localities, having independent pedigree, are comprised in the lot. In the present series individual litters were not divided into a "warm room" and "cold room" half, as had been done with those of the preceding year.

ature conditions will be further discussed after the first table of measurements has been presented. The mean relative humidity in the warm room (12 determinations) was 38.5 per cent, a figure very close to that of the preceding year. The humidity of the cold room (14 determinations) was 76.5 per cent, being thus considerably greater than that of the preceding year. This is accounted for by the fact that the cold room used during the present year has been situated in a building which is entirely unheated.

With respect to weight there is a remarkably close agreement between each of the four averages here presented and the corresponding one of the preceding year. In each year's series, the males are larger in the cold room lot, while the females are larger in the warm room lot. From the magnitude of the probable errors, however, we cannot feel sure of the validity of these differences.<sup>32</sup>

Regarding tail length, it will be noted that the differences, while considerable, are very much smaller than those to be found in Table 3. The warm room males have tails which are 11.8 per cent longer than those of the cold room lot. This difference, in the case of the 1907–1908 lot, was 27.1. The warm room females show a mean tail length which is 15.9 per cent greater than that of the cold room individuals, as compared with a difference of 33 per cent in the earlier lot. Thus, as in the preceding year's measurements, we find that it is the females which have been modified most in this respect. But the amount of modification for each sex has been less than half that which was earlier observed. Regarding variability no deductions of interest are to be drawn. No such evidence of a higher variability in the cold room lot as was previously noted is here to be found. In fact, the reverse is possibly true.

The salient fact brought out by this comparison is the relatively small degree of modification in the tail length of the present lot of animals. This fact is not satisfactorily explained by a comparison of the temperature conditions for the two years. We find, it is true, in the later series a somewhat smaller difference in temperature between the two rooms during the first six weeks of the

<sup>32</sup> See supplementary note below.

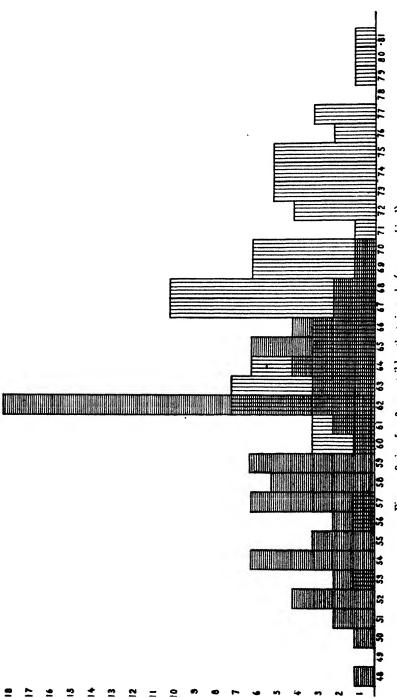


Fig. 14 Series of 1908-1909: tail length at six weeks (sexes combined).

animals' lives. This difference here amounts to 14.5° C., as compared with 16.6° during the winter of 1907–1908; and it might be at once inferred that in this fact we had a key to the difference of results. It must be noted, however, that, while the warm room temperature has been considerably lower for this period, during the later year (19.8° as compared with 23.7°), the cold room temperature has likewise been somewhat lower (5.3° as compared with 7.2°). Now a comparison of Tables 3 and 12 shows us that while the warm room tails agree pretty closely in length in the two series, the cold room tails are much shorter in the earlier one

TABLE 13
Series of 1908-1909: percentages of increase in the interval between successive measurements

-		3 MO	NTH8 OF INCREASE
Weight	Tail	Weight	Tail
. 12.604	67.19 60.11		20.31 ± 0.61 22.79 ± 0.46
. 12.663	68.95 59.49	40.09± : . 40.75± ?	15.81 ± 0.42 18.53 ± 0 66
	Weight	12.604 67.19 13.180 60.11	Weight Tail Weight  12.604 67.19 65.58 ± ? 13.180 60.11 58.86 ± ?

<sup>\*</sup> See Table 12.

Were these modifications simple functions of the temperature differences, we should not have expected such a state of things. Indeed the author has no explanation to offer for the striking difference between the two years' results.

A second set of measurements was made upon the same mice at the age of three months. The statistical treatment of these later figures is not yet quite complete. I have determined, however, the mean percentage increase in weight and tail length for each sex, both in the warm room and the cold room lots. Since every

<sup>&</sup>lt;sup>33</sup> This is the figure for the room in which the thermograph was kept, and in which most of the mice lived during the greater part of this period. For certain reasons, however, all of the animals were kept for a period of varying length (8 to 42 days—the last in the case of only one brood) in another room, having a mean temperature about 4° higher than that of the room first referred to. This fact of course complicates the situation somewhat.

mouse in the present year's experiments is identified by a mark of its own, it has been possible to compute the rate of increase for each animal individually. In Table 13 are presented the mean percentages of increase for each character during the period in question.

It will be seen from this table that in all four cases the figure expressing the increase in a character is larger in that group in which the previous absolute measurement had been smaller. It must be added, however, that in neither case is the figure for weight of any significance in this connection, despite the differences between the averages. The variability in the weight-increase has been enormous (ranging from 9 per cent to 143 per cent), so that the probable errors (not yet computed) are undoubtedly very large. For the growth of the tail, however, the case seems fairly certain. It will be recalled that in the preceding series it was the figures for tail length which bore the strongest testimony to the principle of the leveling down of original differences. Reference to the temperature curves shows that here, as previously, the differences in the conditions have increased rather than diminished during this period. As regards the increase or decrease in variability within each lot, nothing can be said here, since the standard deviations have not been computed for the 3-months measurements.

Supplementary. A yet later series of animals (born March 1909), consisting of a larger number of individuals than any of the preceding lots, has yielded, after similiar teatment, the following results: (1) a difference in tail length somewhat greater than that shown in the preceding series (16.7 per cent in the present case); (2) an indubitable difference (both absolute and relative) in foot length, which applies equally to both sexes and fully confirms the earlier conclusions on this point; (3) a similar difference in ear length, which, however, is of far less certain significance; (4) a difference in weight, both sexes being heavier in the warm room lots (cf. pp. 118, 138 above), 'although this difference was greater for the females (11.3 per cent) than for the males (9.1 per cent). The temperature differences to which the animals were subjected had already begun to diminish at the time of the first measurement (42 days), and both lots were trans-

ferred to the same room when at the mean age of 11 weeks. When measured later, at the mean age of 3 months, the difference in tail length had diminished to 6.7 per cent. The data for this series have not yet been fully compiled.

#### SUMMARY OF STATISTICAL DATA

The more significant facts which may be disentangled from this mass of data may be briefly summarized as follows:

- I The tail length (whether absolute or relative) was found to be very much less in the case of those mice which were reared at the lower temperature.
- 2 This difference was not accompanied by any appreciable difference in the number of vertebræ.
- 3 The foot length was likewise considerably less in all of the cold room lots, though the differences were not so large as in the case of the tail.
- 4 In two series, at least, the mean ear length was found to be smaller at the lower temperature, but these differences are perhaps to be regarded as "accidental."
- 5 Body length was not affected with any degree of certainty; while the influence of temperature upon weight, although evident in certain cases, was inconstant, and seemed to depend upon sex.
- 6 Differences in the average quantity (weight) of hair have been demonstrated for the only series in which the point has been tested. The cold room lot were found to have an average amount of hair which was 11.4 per cent greater absolutely and 13.6 per cent greater relatively (i. e., allowing for the dimensions of the animal) than that of the warm room lot. The number of individuals was, however, small (43) and the variability was high.
- 7 In another experiment a considerable difference in the average number of hairs per unit of skin area was found among the male mice, the number being greater in the cold room individuals. Here, likewise, the high variability and small number of individuals render any conclusions doubtful.
- 8 No constant differences in variability were observable between the warm room and the cold room mice.
  - 9 To what extent the modifications cited above have resulted

from differences in temperature and to what extent from differences in humidity is not certain under the conditions of the experiments.

- 10 Comparisons of earlier and later measurements upon the same animals show that there is a distinct tendency toward the reduction of these experimentally produced differences during subsequent growth, even when the conditions remain unchanged.
- II A diminution in variability within each of the contrasted groups, during the course of growth, was shown to be probable for tail length, at least, and possible in the case of weight.

In order to complete this summary, I will add by way of anticipation:

12 The modifications thus artificially produced are such as have long been known to distinguish northern from southern races of mammals.

#### GENERAL DISCUSSION OF RESULTS

In the following comment upon the results of these studies, I shall commence with the last statement in the summary. It is a most significant fact that the experimentally produced differences which have been discussed in the present paper are found to be of just such a nature as are recognized by mammalologists and ornithologists as distinguishing the northern from the southern representatives (individuals or geographical races) of some species. I. A. Allen has repeatedly called attention to the "marked tendency to enlargement of peripheral parts under high temperature or toward the Tropics." Baird and other writers had previously made incidental mention "of the larger size of the bills of southern representatives of northward ranging species" of birds, but Allen offers some detailed examples of this. 55 He concludes that "an increase in the length of the bill is most frequent in long-billed species, while in short-billed ones the increase is in general size. without material change in its proportions" (p. 231). A greater curvature sometimes accompanies the increase in length. Like-

<sup>&</sup>lt;sup>24</sup> The influence of physical conditions in the genesis of species. Radical Review, I, 1877, pp. 108-140. (Reprinted with annotations in Annual Report of the Smithsonian Institution for 1905.)

<sup>&</sup>lt;sup>20</sup> On the Mammals and Winter Birds of East Florida, etc. Bulletin of the Museum of Comparative Zoölogy, vol. 2, 1871, pp. 161-450, pl. iv-viii.

wise, "there are well-known instances of an increase in the length of the tail" (meaning the tail feathers). Much more explicit statements are offered regarding mammals, both by Allen and by Coues, though it is stated by the former that the responses to climatic conditions are less evident in this class than among the birds. "In mammals which have the external ear largely developed, as the wolves, foxes, some of the deer, and especially the hares, the larger size of this organ in southern as compared with northern individuals of the same species is often strikingly apparent. . . . . In Lepus callotus ('Lepus texianus and its subspecies' -later note], for example, which ranges from Wyoming southward far into Mexico, the ear is about one-fourth to one-third larger in the southern examples than in the northern. . . . Among the domestic races of cattle those of the warm temperate and intertropical regions have much larger and longer horns than those of northern countries. . . . Naturalists have also recorded the existence of larger feet in many of the smaller North American mammalia at the southward than at the northward among individuals of the same species. . . . . "" In his monograph on the Muridæ<sup>37</sup> Coues repeatedly makes similar statements. Referring to a mouse, "Hesperomys leucopus" (now Peromyscus leucopus) (p. 66), he says: "The arctic series averages larger than the United States specimens, and has shorter feet and ears, as well as shorter tail," and he alludes later to "the well-known law of smallness of peripheral parts in Arctic mammals" (p. 83). Comparing the red-backed vole, "Evotomys rutilus gapperi," a more southern "variety," with the species "E. rutilus,"38 he finds that the vertebral part of the tail is, on the average, about a third of an inch longer in the former, while the foot is 72 hundredths of an inch in length, as compared with 70 hundredths of an inch in the northern form. Relatively, the differences are even

<sup>&</sup>lt;sup>26</sup> Op. cit., 1905, pp. 382-384.

<sup>&</sup>lt;sup>37</sup> Monographs of North American Rodentia.—Report of the U. S. Geological Survey, vol. xi, 1877, pp. 1-1091.

<sup>&</sup>lt;sup>38</sup> It is quite possible that two or more distinct species are here referred to. I am not sufficiently familiar with the classification of the Muridæ to know the present status of the various species and varieties referred to by Coues. "E. rutilus gapperi" is now regarded as a true species, Evotomys gapperi.

greater, since the northern animals are of larger size. Indeed, the authors cited dwell with equal emphasis upon the larger size of the northern representatives (individuals or varieties) of species, both of birds and mammals, as compared with the southern. Previously, Allen tells us, Baird had "explicitly announced a general law of geographical variation in size; namely a gradual decrease in size in individuals of the same species with the decrease in the latitude and altitude of their birthplaces.<sup>39</sup> And Allen further affirms that "this is true not only of the individual representatives of each species, but generally the largest species of each genus and family are northern.<sup>40</sup>

The foregoing statements were made before the days of exact biometry, and an examination of the tables of measurements offered us shows that in most cases they comprise relatively few individuals and that the material used was not homogeneous, i. e., it includes alcoholic and fresh specimens, as well as dried skins. For this reason most of these tables are not likely to be of very great use to the modern student of variation. In more recent years, a very extensive mass of similar measurements has been gathered by a considerable number of naturalists, but, so far as the writer has been able to discover few if any of these have been subjected to statistical treatment with reference to testing the generalizations of Baird, Allen and Coues.<sup>41</sup> It would seem overskeptical, however, to reject the emphatic opinions of a number of able naturalists upon these matters, particularly as we have no more satisfactory data at our disposal.

Regarding the pelage, there can be little doubt that this likewise responds directly or indirectly to climatic conditions. "At the northward, in individuals of the same species, the hairs are longer and softer, the under fur more abundant, and the ears and the soles of the feet better clothed. This is not only true of individuals of the same species, but of northern species collectively, as compared with their nearest southern allies." Both Coues

<sup>39</sup> Op. cit., 1871, p. 230.

<sup>40 1905,</sup> p. 378.

<sup>&</sup>lt;sup>41</sup> I state this on the authority of several of our leading students of mammalian distribution to whom I have appealed for information.

<sup>42</sup> Allen, 1905, p. 382.

and Allen cited many specific instances of this fact for mice, hares, squirrels and other rodents. Moreover, obvious seasonal changes are to be observed in some species. Speaking of the squirrel, Sciurus hudsonius, var. hudsonius, Allen says: "In summer the soles of the feet are naked, often wholly so to the heel; in winter they are wholly thickly furred, only the tubercles at the base of the toes being naked. The general pelage is also much fuller, longer and softer in winter than in summer."45

It may well be that the change in the quantity of hair which appears to have been produced in the white mice during the experiments above described was comparable to these seasonal changes, i. e., that the results were purely temporary, and would have disappeared with a cessation of the conditions employed.44 Indeed, since the life of an individual hair is comparatively brief, it would be necessary to effect some permanent change in the physiological activity of the hair follicles, in order that differences such as these should endure. Whether or not these effects are permanent, it has been believed by many that various changes in the character of the hair coat occur in domestic animals as the result of transferrence to an unaccustomed habitat. Darwin, indeed, tells us45 that "Great heat, however, seems to act directly on the fleece; several accounts have been published of the changes which sheep imported from Europe undergo in the West Indies. Dr. Nicholson of Antigua informs me that, after the third generation, the wool disappears from the whole body, except over the loins; and the animal then appears like a goat with a dirty door-mat on its back." And again: "It has been asserted on good authority [Isidore Geoffroy St. Hilaire] that horses kept during several years in the deep coal-mines of Belgium become covered with velvety hair, almost like that on the mole." The "classical" Porto Santo rabbit may be cited as another and perhaps more authentic instance of the modification of mammals through changed cli-

<sup>48</sup> Monographs of N. A. Rodentia, p. 675.

<sup>44</sup> It is uncertain, to be sure, in how far the season changes of the hair coat of mammals are direct responses to climatic conditions.

<sup>4</sup> Variation of Animals and Plants under Domestication, vol. i, p. 124.

<sup>&</sup>quot; Op. cit., vol. ii, p. 336.

matic conditions. Here, not only the hair, but other features, were affected.

So far as the present writer is aware, however, no such differences as have formed the principal theme of this paper have been previously brought about by direct experiment or even produced under such circumstances as would warrant one in stating positively that they were the immediate results of external conditions. Lydekker, in the work already referred to, cites a case on the authority of "an American newspaper" (so notoriously infallible in matters scientific!) which would certainly be important if true. It is worthy of mention only because the modifications alleged accord so well, in some respects, with those which have been demonstrated for mice. In order to combat the rats in a cold-storage warehouse at Pittsburgh—so the story runs—cats were introduced. The first of these died. "One cat was finally introduced . . . . which was able to withstand the low temperature. She was a cat of unusually thick fur, and she thrived and grew fat in quarters where the temperature was below 30°. By carfeul nursing, a brood of seven kittens was developed in the warehouse into sturdy thick-furred cats that loved an Icelandic climate. They have been distributed among the other cold-storage warehouses of Pittsburgh, and have created a peculiar breed of cats, adapted to the conditions under which they must exist to find their prey. These cats are short-tailed [italics mine], chubby pussies, with hair as thick and full of under-fur as the wild cats of the Canadian woods. One of the remarkable things about them is the development of their 'feelers.' . . . . In the cold warehouses the feelers grow to a length of five and six inches. This is probably because the light is dim in these places, and all movements must be the result of the feeling sense."

I am informed by Dr. A. E. Ortmann, who has kindly taken the trouble to make some inquiries regarding this story, that he can find no foundation for it whatever. Those who had heard of it at all did not take it seriously. Moreover, as Dr. Ortmann points out, it seems quite unlikely that cats could be forced to live in a cold-storage warehouse unless caged. It has, nevertheless, seemed worth while to cite this account, owing to the prominence given to it by Lydekker.

Passing to the question of the adaptiveness of these experimentally produced modifications, that of the hair would surely seem to fall within this category. A complementary physiological explanation for the change would doubtless be likewise possible, had we a sufficient knowledge of the various processes concerned. The shrinkage or "drawing in" of the peripheral parts under the influence of a cold climate might also be regarded as adaptive, for the reduction of these thinly clothed surfaces would diminish, at least theoretically, the radiation of heat from the body. again a simple physiological explanation is likewise possible. We might either appeal to the effect upon the peripheral circulation (as does Allen) or to the direct influence of temperature upon the protoplasm of the growing parts. In the case of the feet of the mice in the above experiments, the greater activity of the young animals in the warm room, and the greater consequent exercise of the limbs, may possibly have played some part in bringing about the difference.

To what degree the modifications which I have described have been due to temperature and to what degree they have been due to humidity is not clear under the conditions of the experiments. As has been stated, the two have varied inversely. Allen and Coues seem to regard such differences, when presented by mammals in nature, as due chiefly to the temperature factor. Nevertheless, the former writer tells us, speaking of hares, that "there is also a marked tendency to an enlargement of the ears in proportion to the aridity of the habitat. . . . . In this connection, also, attention may be called to the fact that all of the long-eared that the color of the pelage of mammals and that of the plumage of birds is well known to vary with the hygrometric conditions. In many species of birds the degree of pigmentation is said to be a function of the mean humidity of the habitat. Tower, indeed, regards the humidity as being much more important than the temperature in the production of color changes in beetles. Until,

<sup>47</sup> Monographs of N. A. Rodentia, p. 272.

therefore, it is possible to separate these two factors in our experiments, we cannot state with any certainty to what degree each has been operative. A priori, it would seem, perhaps, that the changes in the mice have been such as could more reasonably be attributed to temperature.

The fact that the same sort of differences as those which sometimes obtain in nature between northern and southern species or varieties of animals have been produced by artificial conditions acting within the individual lifetime will be taken by some as evidence that these differences in nature are likewise entirely "ontogenetic" or acquired independently by each individual. Conversely, the neo-Lamarckian will perhaps argue—and with equal right—that here we have evidence that natural varieties and species have resulted from the accumulated effects of external conditions since the reality of such effects has been palpably demonstrated by the present experiments. Neither conclusion is justified by the facts before us. It remains to be settled experimentally (and thus only!) whether or not such modifications are transmissible.

It has already been stated that no constant difference in size between the warm room and cold room individuals has been found to obtain throughout my series. Here, then, the reputed effects of natural climatic conditions have not been paralleled. It is quite possible that the cold was so severe during the early growth stages that some individuals were stunted. Indeed it has been pointed out for the 1907-1908 series that there was considerable mortality amongt the cold room lot in early life. Reference to the frequency polygons in Fig. 2 shows that there are two distinct modes among the cold room individuals; and I have determined that this is equally true of each of the sexes taken separately. The impression conveyed is that there are two pretty distinct groups, one of which was stunted by exposure to the cold, the other being favorably affected, so as even to surpass the warm room lot in size. It must be added, however, that no such effect is manifest in the 1908-1909 series.

One of the most important general conclusions which seem warranted from an analysis of the foregoing results is the principle of the levelling down of experimentally produced inequali-

ties, even while the conditions which gave rise to them remain in full force. A diminution in initial differences of size has been demonstrated by Minot<sup>48</sup> in the case of growing guinea-pigs. His findings upon this point are thus summed up: "The study of the individual variations yields two important conclusions: First, that any irregularity in the growth of an individual tends to be followed by an opposite compensating irregularity. Second, the variability diminishes with the age." Thus, "if an individual grows for a period excessively fast, there immediately follows a period of slower growth, and vice versa, those that remain behind for a time, if they remain in good health, make up the loss (at least in great part if not always completely) soon after. . . . is probable that the same is true for man and that therefore the usual and even the severer illnesses of childhood and youth do not greatly affect the ultimate size of the adult." Pearson,40 likewise, has shown that the variability both of weight and of stature in man diminishes from infancy to adult life. And indeed it is a matter of common experience that an early handicap in the size or strength of a child is frequently "outgrown," wholly or in part.

The variability which the above-named writers have considered is doubtless in part due to blastogenic differences, in part to somatogenic ones, resulting from fetal or post-natal conditions of nutrition, etc. In my own results, however, the most noteworthy fact is not a reduction in the general variability of my stock, but the diminution of differences whose cause is known to be external—and this while the effective conditions remain unchanged. The foregoing statement applies to the growth of the tail, both of the male and the female mice, between the age of six weeks and the age of  $2\frac{1}{2}$  (or 3) months. It likewise holds, with some qualification, for the growth of the tail during the next interval between the measurements, i. e., between  $2\frac{1}{2}$  and 7 months. In the latter case, however, the data are fewer, and the allowance is necessary that about midway during this third period the temperature conditions were equalized

<sup>48</sup> Senescence and Rejuvenation. Journal of Physiology, vol. xii, no, 2. 1891, pp. 97-153, pl. ii-iv.

\*\* Proceedings of the Royal Society, Irvi, 1900, p. 32 (rited by Vernon, in "Variation in Animals.")

<sup>\*\*</sup> Proceedings of the Royal Society, Ixvi, 1900, p. 23 (cited by Vernon, in "Variation in Animals and Plants").

for the entire lot of animals. The diminution of differences in weight between the contrasted groups of animals is less certain, though it appears tolerably clear in the case of the 1907–1908 females. It will be recalled, however, that the differences in weight were only very doubtfully regarded as results of the temperature conditions.

This tendency toward a reduction of experimentally produced differences in the relative size of parts, should it prove to be general, is of considerable theoretic interest. It adds another to the many well-known examples of a "regulative" tendency in living things. 50 After the initial shock of change, with its resulting effect in deflecting the organism away from its individual norm, there would seem to be a continuous effort to regain the latter. Here we have a principle which might be said to bear the same relation to individual growth as Galton's "law of filial regression" bears to stem-history, though the analogy may be merely superficial. In either case, however, we have to do with a "reversion to mediocrity." The process in question is directly opposed to that conceived of by Weismann, in his theory of "germinal selection," as occur-ring among the determinants of the germ-plasm. According to this hypothesis, a given determinant, if once handicapped by unfavorable nutrition, is more and more pushed to the wall by its more fortunate competitors until it may be totally annihilated. The disappearance of useless structures in phylogeny and the frequent orthogenetic trend of evolution is thus explained. If it be objected that this analogy of mine is out of place I can only reply that Weismann's whole conception of a struggle among the determinants of the germ-plasm was derived from what was assumed to occur among the parts of the organism as a whole. Some evidence has been offered above for the existence of a tendency in the growing body quite at variance with the demands of that theory. To many readers, on the other hand, it will doubt-

<sup>&</sup>lt;sup>50</sup> Vernon's principle that "the permanent effect of environment on the growth of a developing organism diminishes rapidly and regularly from the time of impregnation onwards" (op. cit., p. 199) would account for the failure of these differences to augment with the growth of the organism. But it certainly would not in itself account for the absolutely greater increase shown by the more retarded organs (or organisms) mentioned on p. 137 above.

less seem quite frivolous to attempt any serious refutation of the "germinal selection" hypothesis. In justification, I will but call attention to the fact that this theory is not only treated respectfully but is ably defended in the most recent general treatise on heredity.<sup>31</sup>

<sup>&</sup>lt;sup>81</sup> I refer to Thompson's admirable work, Heredity (G. P. Putnam's Sons, 1908), which should be in the hands of every student of this province.

# FURTHER OBSERVATIONS ON THE BEHAVIOR OF TUBICOLOUS ANNELIDS<sup>1</sup>

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#### CHARLES W. HARGITT

In a former contribution on the general subject above stated the present writer ('06)<sup>2</sup> described certain observations and experiments on several species of these interesting annelids. During the past summer these have been variously repeated and extended, and it is the purpose of the present paper to supplement the account given in the former by such additional facts as have been brought to light, and thus afford a more complete account of the behavior of these annelids than was made by the earlier contribution. The present account has to do with but one species, namely, Hydroides dianthus, except as there may be incidental occasion to refer briefly to others.

The earlier observations were made entirely upon specimens kept in the aquarium. In the following account considerable emphasis will be placed upon observations made on specimens in their native habitat, and on the conclusions drawn from modes of behavior exhibited by tubes taken at various localities, and under greatly differing environments. It seems worth while to emphasize this point, as it is altogether probable that certain of our experimental results obtained from animals in cages, finger-bowls, etc., have given rise to more or less inadequate, if not misleading and erroneous conclusions. For example, it will be found in phases of the accounts which follow that the behavior of specimens taken from depths of ten to twenty fathoms shows considerable differences as compared with that shown by specimens from shore waters, or from depths of two or three fathoms. Similarly the aspects of growth in specimens taken from rocky bottoms will

<sup>&</sup>lt;sup>1</sup> Contributions from the Zoölogical Laboratory, Syracuse University.

<sup>&</sup>lt;sup>2</sup> Journ. Exp. Zoöl., vol. iii, p. 295.

show important contrasts when compared with those taken from muddy bays, or mouths of rivers.

Again, in the former paper attention was directed chiefly to the reaction of specimens to light, and that too without particular efforts to test the variation or modifiability of behavior under slightly differing conditions. In the present account will be shown a much wider range of tests, and some particular results as to individual differences of behavior not given before, as well as evidences of modifiability to which only incidental reference was made.

Most of the experiments and observations which follow were prompted by some results obtained from a series of specimens taken from a depth of some twenty fathoms off Gay Head in a dredging expedition made by the Fisheries steamer, Fish Hawk, early in August, 1906. These colonies were brought to the laboratory along with other material obtained. Upon arrival the specimens were placed as usual in shallow aquaria containing freshly obtained sea-water and left for some time in order that they might expand and thus be ready for the usual tests. One of the first points of interest noted was the unusual slowness of the worms to emerge from the tubes. And when finally there were some signs of activity they yet seemed extremely wary, protruding only the merest tips of the gills, and frequently retracting them as if in fear. This I took for a time to be due chiefly to the probable effects of the rough handling incident to the operation of the dredge and their subsequent transfer from vessel to vessel, etc.

After some hours they at last became fairly expanded in the usual manner, though not fully. And when finally the tests with shadows were made they showed, to my great surprise, wholly negative results. They were left again undisturbed for some time and then further tested in the same way only to again fail to show any sensory irritability. They were then left till night, when they were tested with the electric light as in the experiments of the former year<sup>3</sup> and here again the results were almost entirely negative. This seemed so wholly peculiar, and so utterly contradictory of the almost uniformly positive reactions of former

<sup>&</sup>lt;sup>a</sup> Cf. op. cit., p. 300.

experiments that I was greatly puzzled. The specimens were left until the following day when the tests were repeated, and with similar results though with considerable variations among individuals.

It occurred to me that these strangely negative results might be in some manner related to the unusual depths from which they had been taken. Accordingly I secured a single specimen from about the docks, and hence from an unusually shallow habitat, and placed it in the same aquarium with the others. This specimen behaved quite as had those of the former year, protruding its crown of gills promptly, and showed the same prompt and constant response to shadow stimuli as had been observed formerly.

This suggested at once a series of comparative studies of specimens taken from various depths. The results of these experiments are shown in part in the accompanying tables to which detailed reference will be made slightly later. Variously tested both by artificial and natural light as before, the reactions of the shallow water specimen were incomparably more acute and constant than any of the specimens from the deeper waters.

In order to secure a more critical comparison of the relative sensory qualities of these specimens they were arranged in an aquarium on my laboratory table so placed in relation to both natural and artificial illumination as to enable one to easily control either, as to relative intensity, at will. To the ordinary shadow tests produced by the interposition of a screen as in the previous experiments, the behavior showed the same diversities as have already been noticed. In order to have the tests as nearly identical as possible in character, and at the same time within easy control, it was decided to use the stimulus of the suddenly extinguished electric lamp, as referred to above. By this means it was quite easy to have all the specimens in similar conditions of expansion when a test was made, and by the ease with which the light might be extinguished, and the equal ease by which it could be turned on again almost instantly, one may clearly determine at a glance what specimens had responded and any which had failed to so react.

Usually a given interval was allowed to intervene between any

two tests. This interval was, on the average, about five minutes, sometimes less, or slightly more, as conditions might require. Of the signs employed in the tables, namely, plus, minus, and zero, the first two indicate simply positive and negative reactions of the specimens, i. e., retraction into the tube or failure to retract. That of zero signifies that in a given test the specimen so designated was out of commission or, in other words, had withdrawn into its tube at the time the test was made. This was not frequent, but as will be seen it happened occasionally with different specimens. The letters A, B, C, etc., arbitrarily indicate the record of given specimens throughout the series of experiments of a given table. The numbers at the left of the tables indicate the number of the tests of a given day or time.

As a check against possible error which might be involved in a too rigid attention to results obtained from laboratory experiments some pains were taken to observe the behavior of specimens in their natural habitat. This was possible by selecting specimens to be found along the lower tide lines and in tide pools, and at favorable times when the surface was smooth on still days, thus making observation practicable, tests were applied similar to those employed in the laboratory. Without taking time for extended details it may suffice to state that the reactions obtained from shadow and tactile stimuli were essentially the same as the former, and confirmed them in almost every particular. differences as to individual reactions were perceptible, especially as to tactile responses. This I am disposed to attribute to the peculiarity of the habitat. It seems not unlikely that the effects of the buffeting of wave action to which these specimens are more or less subject may account for these apparent differences in tactile reaction.

Another feature was apparently quite different in the shore specimens, namely, the close adherence of the tubes to the substratum on which they grew. There was no tendency to grow upright and free, as was the case in those from deep waters. And this again may be interpreted as a further expression of the conditions involved in the environment. It would be quite impossible for the tubes to withstand any such buffetings as the waves and pound-

ing surf are constantly imposing upon objects under these conditions. The tubes show throughout the closest adherence to the rocks, which in this environment forms the almost exclusive base of support, though one sometimes finds specimens attached to the shells of living snails occupying these pools. When in the process of growth the worm comes to a sharp angle of the rock it either turns and grows back upon itself, or turns sharply over the edge and along the opposite surface. In these conditions the tubes show a distinctly flattened surface along the line of contact which is hardly perceptible on those from deeper waters. All these facts lend still further confirmation of the suggestion of adaptation as the more probable explanations of the facts, rather than the more usual explanation of stereotropism, though the latter may not be wholly lacking.

As already intimated in the beginning, pains were taken to secure specimens from as wide a range of depth and habitat as possible in order to detect any peculiarity of behavior which might be due to purely local, or environmental conditions. From resulting comparisons marked differences were distinguishable in specimens dredged from a muddy bottom and those from rocky or sandy bottoms. The former tended to grow in larger colonies, and in a more or less vertical aspect, while the latter were often isolated and independent, and closely adherent throughout to the substratum, as much as those to be found in tide pools or shore lines, referred to above. The extreme contrast was found in colonies taken from the debris about river mouths, or enclosed bays which received the drainage or sewage of adjacent towns. In these conditions colonies of immense size were found, made up of hundreds, or even thousands, of specimens. In some cases these aggregations were apparently of considerable age and formed massive calcareous accumulations, not greatly unlike coral heads in sub-tropical harbors. Occasionally colonies would be found on the most unexpected substratum. For example, the writer was shown one such taken by Mr. Geo. M. Gray at the mouth of the New Bedford river growing on an old granite kettle, covering it almost completely, both inside and out, and forming a most picturesque object, especially when the hundreds of specimens of various size

and color were fully expanded in the aquarium. Some further reference to this colony will be made in another connection.

A point of more than passing moment is the fact that in almost every colony of these annelids, whether large or small, were to be found evident individual differences as to sensory response. In most cases there was more or less sharpness and uniformity as to reaction, while in others there was just as evident a degree of uncertainty and inferiority of response. And the same variation was evident in the matter of recovery time following a stimulus. In some individuals recovery was prompt and constant, while in others it was just the opposite, sluggish and uncertain. Again, many specimens react variously on different occasions. On some days there were noticeable and measurable differences of reaction to a given stimulus. Many of these facts are graphically portrayed in detail in the several tables, in connection with which they are briefly discussed. Attention may be called particularly to the striking individuality exhibited by specimens and colonies taken from different habitats and localities. And the wider one's range of observation the more evident and significant does this feature become.

#### PHOTIC EXPERIMENTS AND FATIGUE

The following individual records of the reactions of three specimens tested under exactly the same conditions may serve to further illustrate the point here under consideration and certain other features of similar character. The specimens were taken just below tide line on the adjacent shore of Buzzards Bay on July 20, and for convenience are designated as A, B and C.

The specimen A was tested by the shadow stimulus at intervals of one minute, and for fifteen times in succession. In every case there was a prompt and positive response, the creature withdrawing into its tube. Protrusion was somewhat variable as to the time concerned, varying from ten to thirty-five seconds, but the results were unmistakably decisive in every instance.

In the case of B the tests were applied at intervals of 45 seconds, and, as before, fifteen times in succession. In this experiment there happened to be five specimens in a colony, and taken as a

whole there was possible a total of 75 reactions. Of this number 71 positive responses were recorded, with four negative ones, or 94.5 per cent as compared with the 100 per cent of A.

Specimen C was tested as in the former cases, but at intervals of 30 seconds, and carried on beyond the fifteen tests of the former, with a view to determine at what point any signs of fatigue might be detected, or whether it was possible to distinguish any periodic recurrence of negative reactions. For twenty-five tests the specimen responded promptly and positively. Following this reaction it remained in the tube for a period of exactly five minutes. At the end of this period it protruded and was promptly tested as before. At the eighth test the response was only partial. At the seventeenth test it failed to respond, but continued after this to react positively up to and including the twenty-ninth. The thirtieth was negative, as was also the thirty-second. The thirtythird test was followed by a positive response in which the specimen again remained in the tube for a period of five minutes. On emergence the tests were resumed and with the following records: The second and sixth responses were only partial withdrawals, the tenth and thirteenth tests failed to induce response; fourteenth, partial. The fifteenth, sixteenth, eighteenth, twenty-first, twentythird, twenty-fourth and twenty-fifth were all negative.

In this series of tests it seems strongly probable that we have something quite analogous to fatigue, especially in the final twenty-five tests. At any rate some condition had become operative in modifying in a marked degree the behavior of C as compared with A and B and the earlier ones of C. The fact that the tests were applied at very brief intervals, and, moreover, that they were long continued, go to strengthen the suggestion of fatigue. It is necessary, however, in this connection to point out that fatigue as here employed does not necessarily imply exhaustion of the bodily musculature of the worm, though this may be in some measure involved. But fatigue may be sensory, a condition analogous to similar conditions induced by long-continued direction of the vision to a given test. This point has been referred to in the previous paper<sup>4</sup> and has also been noted by several earlier

<sup>4</sup> Op. cit., p. 302.

observers, among them Patten, whose comment was cited in the previous paper (p. 312).

In an interesting article on the behavior of these annelids Ada W. Yerkes<sup>5</sup> has expressed doubt as to the question of fatigue, but seems to have misinterpreted the significance of the term as employed in my paper, and has, I think, misapprehended my conclusions. For example, I did not attribute the failure to react under rhythmic stimuli to fatigue, as Mrs. Yerkes has suggested. What I did say was, "In connection with this matter of rhythmic shadows it was observed that where experiments were repeated with any considerable frequency specimens sooner or later became somewhat irresponsive, often failing entirely to react to any of the usual tests. This I am inclined to regard as the result of fatigue" (p. 301). Concerning the failure of response under rhythmic stimuli my words were: "May it not be possible that in these rhythmic shadows we have a simulation of the more or less rhythmic shadows resulting from the ripples of wave action." As will be seen, therefore, my suggestion of fatigue had no reference to the matter of rhythm, but to the long-continued experimentation which was designed to induce reactions repeated up to the point of fatigue. Furthermore, it was explicitly stated that under the normal conditions of rhythmic wave action such shadows might be assumed to have ceased to act as definite stimuli, the creatures having become accustomed to their effects by reason of their constancy. It seems to the writer that the above account of later experiments, with others of similar nature, goes far to confirm the earlier view. As one carefully observes such behavior it is difficult to escape the conviction that something akin to fatigue of some sort is involved. It may be that certain sensory cells only are affected, or it may be that certain central ganglia are involved, whose cells have to do with sensory and muscular coördination.

Some recent work on the problem of fatigue in nerve cells by Drs. Smallwood and Rogers has thrown new light on the subject and furnishes convincing evidence as to changes in the cells them-

<sup>&</sup>lt;sup>5</sup> Jour. Comp. Neurol. and Psychol., vol. xvi, p. 442.

Jour. Comp Neurol. and Psychol., vol. xviii, p. 67, 1908.

selves associated with experiments on fatigue. Some of their results are similar to phases of those here under review, and seem to confirm the suggestion of fatigue.

#### TACTILE EXPERIMENTS

In the former paper reference was made in several places to tactile reactions, but no details of experiments were given. These I have subsequently repeated and extended and with such variations as to suggest certain inferences and conclusions not previously considered.

As might naturally be expected in creatures whose sensory powers are as delicately adjusted as the reactions to light already described, the tactile sense is also very acute. And interestingly enough it is found most highly developed in the gill filaments, the organs concerned with photic sensibility, one and the same organ therefore serving the double sensory function of touch and photic perception. But this is not peculiar to these organisms. Not a few of the lower invertebrates show very similar conditions, and suggest the inference that tactile and visual senses are more or less intimately correlated. Indeed, but for the conventional definitions of these senses, based to a large extent on the highly differentiated organs of higher organisms, it might be fairly allowable to regard them as modified expressions of sensory processes due to stimuli of fundamentally similar nature. No time can be taken in this connection to follow out the suggestion further, but it seems well to call attention to the facts, barely hinting at the more or less obvious inferences concerned.

While experimenting with shadow tests it was observed that now and then a specimen was found whose reactions were markedly inferior, or lacking entirely at times. Something of this will be noticed in the tables. In order to determine that in such cases the behavior was not due to some abnormal or pathologic condition the specimens were subjected to tactile tests, and in almost every case were found to respond as promptly to such treatment as any of the others. And in not a few such cases it was found that a few tactile tests sufficed to awaken, as it were, the dormant pho-

tic sense, the specimen responding subsequently almost as readily and sharply as others. This was particularly the case where some pains were taken to apply the two stimuli successively for a few times. Something akin to this was found in the earlier experiments where attempts were made to excise the gills (p. 304).

## Individuality

In this class of experiments there was evident the same marked individuality of behavior as has already been shown in previous sections. For example, a given specimen, X, was found to be unusually indifferent to slight tactile stimuli. At one time its gills were gently stroked 123 times, some of the latter ones quite vigorous, before it finally retracted within its tube. The same specimen at a later hour the same day was tested again. At the first touch it responded with a sharp and decisive retraction. After protruding it was again touched with a glass rod, and at the second test again retracted. The third time it retracted after the fifth test. The fourth time only after the twelfth. The fifth time it only responded after the 115th stroke of the rod. On the sixth test it responded after the thirty-seventh stroke; and in the seventh test only retracted after the gills had been stroked 237 times, some of which were decidedly vigorous, and even then the reaction was' not sharp or violent as in some cases.

An interesting feature was observed during the progress of these experiments, namely, the exhibition of what seemed to be a definite sense of tactile discrimination. While observing a group of specimens it was noticed that those occupying adjacent tubes often in the act of protrusion thrust their gills against one already expanded, but without in any way causing a retractile response on the part of either specimen. It was also observed that a specimen of Sabella living among the same colony occasionally thrust its body out of the tube for considerable distance and swayed it laterally and variously, jostling the gills of Hydroides in the process, but with the same negative results as have been referred to in the previous case. To make the test still more explicit a specimen of Sabella was removed from its tube and, being held with forceps was used as a tactile brush, the gills of other species were brushed

more or less vigorously and somewhat indiscriminately, and again with substantially the same results as just cited. I then tried a somewhat different test, namely, forming a brush out of a tuft of very delicate red alga; this was used as a tactile instrument. While it was possible by extreme delicacy of touch to gently jostle the gills of specimens, there was an appreciable difference as compared with that associated with the foregoing. It was also found that by using a gentle current of sea water ejected from a pipette against the gills the specimens would frequently bear considerable disturbance without reactions. An attempt was made by this means to train specimens to allow similar jostling by means of a delicate glass rod, ejecting water from a pipette in one hand and with the other touch the gills with rod. But it was of small avail. In almost every instance the slightest touch of the foreign body induced the usual sharp response of retraction.

It would seem, therefore, that we have in this type of behavior an example of tactile discrimination of a qualitative nature, enabling them to distinguish as to the nature of the stimulus. It should be noted, however, that in this matter as in that of the shadow stimulus, there was marked individual variation of behavior, and furthermore that these differences varied more or less from day to day.

It was pointed out in the former paper that following certain experiments a degree of shyness or caution was induced. The same thing was quite as marked in the tactile experiments here under review. And in the present case the tests were made with smooth and delicate rods which involved not the slightest injury, such as was involved by the clipping of gills with scissors; still there was almost invariably involved the development of a degree of caution which was quite marked. Following a given test the specimen would protrude only the tips of the gills, followed by a pause; then a further protrusion, then another pause, and often a slight retraction; finally a further protrusion and the expansion of the crown of gills as usual.

#### REACTIONS OF NAKED WORMS

Both tactile and photic tests were made upon specimens which had been removed from the tubes, with a view to determine something of the character of the responses under such radically changed conditions, and also to ascertain the relative sensitiveness of various regions of the body. The earlier experiments had sufficed to determine that the photic sense was limited to the gill regions. It remained, however, to determine whether there was any similar limits as to tactile sensibility. This was attempted by gently touching the body with the blunt end of a delicate rod. As might have been anticipated, it was found that the gills were by far the most highly sensitive. Next to this the immediate region of the head was more or less responsive. The mantle-like portion was found to be but slightly sensitive to touch, and the posterior, free margin of this organ was slightly if at all sensitive to any ordinary tactile stimulation. This was likewise the case with almost the entire posterior portion of the body, only the prick of a needle or the pinch of a forceps inducing some slight muscular reaction.

Touching the gills always aroused a prompt and vigorous reaction. If stimuli were applied rapidly, at five to ten seconds intervals for a few minutes the specimens evidently became fatigued, and soon failed to respond, or did so but feebly, the body having gone into a condition of muscular contraction resembling tetanus. This was followed by apparent efforts on the part of the specimen to relax, the gills expanding in spite of continued tactile agitation. This condition may have been due to something like a convulsion resulting from overstimulation, and perhaps induced in some measure by the unusual condition of nakedness. After a rest of from five to ten minutes the specimen would again respond in exactly the same way, but became sooner unresponsive, lapsing into the same condition of fatigue as before.

Reactions to the shadow stimulus were more or less peculiar and uncertain. This was doubtless due in part to the almost constant writhing and twisting of the body under these conditions involving what I have previously termed "mixed stimuli" (pp. 303, 316). The very unusual conditions of nakedness and help-

lessness involved were such as to preclude anything like simple or direct reactions to any given stimulus. In other words, the conditions involved the operation of a complex of peculiar and anomalous stimuli, so that the reaction to the shadow stimulus was only one of several acting upon the creature at a given time. However, taking a specimen when less disturbed by promiscuous movements of the body it was found that a sudden shadow often caused a sudden response, or jerk of the body, and a correlated contraction of the gills. This might recur upon a second, or even a third test, but after that it became practically indifferent. Repeated after an interval of a few minutes a similar reaction was liable, but failure was also frequent. Excision of the gills of such specimens was invariably followed by total absence of photic reaction, and fully confirm the earlier experiments and conclusions.

Some connected account of the facts portrayed in the several tables in addition to references made at various places in the paper may make clearer their significance and importance. Incidentally it may be stated that these data are but a small fraction of a very large number of observations made during two successive summers. To attempt to include all would unnecessarily cumber the paper with details which, while not without some value, are not vital to the fundamental aims of the investigation.

Tables I and II show records of two series of specimens: A B C D E and H I J K L. Both were from deep water, eight to fifteen fathoms, and show a fair average of reaction of specimens from this environment. The records are specific, that is, made from definite individuals. The really significant feature of these records is their almost exclusively negative character. An interesting detail of Table I is the zero record of specimen C, quite marked in the first, or 11 o'clock test, and its almost exclusively zero record for the 2 o'clock test. In this respect the record is somewhat unusual. A specimen may often exhibit such a behavior at one time and an altogether different aspect at a subsequent test.

In marked contrast with the foregoing is the record shown in Table III. Another series of five specimens, A B C D E, taken from shallow habitat illustrate what hundreds of similar tests of

specimens from similar surroundings have confirmed, namely, the unmistakable predominance of positive reactions to the shadow test. Tables IV and V show the same thing, and also show the rather remarkable variation of recovery time at different tests by the same, and different individuals. For example, in Table IV the recovery time of F and G at the very first test is in extreme contrast, and at the ninth test almost the same contrast, but in

TABLE 1
Showing reactions of specimens from deep waters

	August 9, 11 a.m. Temperature 22° C.				August 9, 2 p.m. Temperature 22.5° C.					
	A	В	С	D	Е	A	В		D	E
1 .		-			_	_	1		-	
2		-				-	- :		-	-
3	_	+10	_	-	_	_	+18	-	+30	+20
4	-	0	-	-	_	<u> </u>		+	_	-
5 .	-	0	+	+	-	! -	- 1	0	i -	, -
6		-	0	0	-	_	-	0		-
7 .	+12	+10	0	0	-	i –	' -	0		-
8		l – i	0			-	-	0	-	-
9	-		0		-		- i	٥	<u> </u>	-
10 .		+	0	-		-	-	0		-
11		0	0	- ,	-	-	+35	0	<u> </u>	-
12 .		-	0	•-		i	'	0	<u>'</u>	-
13 .		-	0			-	;	0		-
14		-				i -	}	0	ì -	-
15		-				-	(	٥	-	_
16		-		_	-	-	, !	0	-	<u> </u>
17	-	-	_		-	i –	(	٥	-	-
18	-	-	-	` <b>-</b> -		-		0	-	-
19		j -	-			ľ –	_	0	-	-
20		-	_	_	-	-	, -	0	-	-
1	_			·		<u> </u>	i		1	1

reversed order. The record of the same specimens at 2 p.m. shows much less contrast in these points, though they are not lacking. In some cases the contrast between certain individuals was much greater than these. In one case a specimen after a given test retracting and remaining in its tube for nearly half an hour, in exact time twenty-six minutes. It does not seem possible to explain these as in any wise due to anything in the nature of the stimulus.

Table VI contains a brief record of the comparative reaction of two specimens to shadow and tactile stimuli. The longer recovery time following the tactile test is naturally what might have been expected. In other records similar conditions were found to those alluded to in citing the contrasts shown in Table IV, and probably explainable on same general assumption.

But there is more involved in the records of these tables than

TABLE II

Showing reactions of specimens H, I, J, K, L, from deep waters

	August 10, 11 a.m. Temperature 21° C.						ist 10, 1:4 <u>:</u> perature 2			
	Н	1	J	К	L	Н	1	J.	K	L
ı		+ 15	-	-	+15	_	_	_	+10	+12
2 .			-	-	-	-		+10	+10	-
3 · · ·	-		-			_	+15	-		
4 .	+25	+ 25	•	+ 12	-	-	-	+ 9	-	-
5	+40	-	+13	+13	-		-		_	
6 .		***			-		-	-		-
7 .	-		-	-	-	_	-		-	
8 .	+ 28	-	+10	-	-	_	-	+10		+ 10
9 .	-		+ 10	-				+11	-	
10 .		_				_		-	-	-
11			-		-			-	-	-
12		-				-	••		-	_
13					-	_	-			-
14 .	-	-	_			-	-		-	_
15'		+ 10	••	-		_		_	-	-
16 .		-	***		-	_		+ 8	-	-
17 .		_		-		0	-			-
18			-	-	-	0		_		-
19					-	_			-	_
20			_	-	-	-	_	_	_	_
					,					

the simple facts of the reactions. As expressions of behavior the facts call for explanation and interpretation. A point of more than passing significance is that involved in the recovery time indicated in the various tables. Does the feature of protrusion, following a given retraction induced by shadow or tactile stimulus, sustain any such relation thereto as to warrant the conclusion that these several aspects are essentially parts of a common reaction

water, 22° C.

cycle? If so should there not be discoverable some definite law of relational sequence?

On the assumption that organisms of this type are definitely organized mechanisms adjusted to the normal play of chemicophysical stimuli there can hardly be hesitation in answering affirmatively the several queries. Organic machines of such character, and designed to illustrate just such principles, are well known in

TABLE III

Showing reactions of specimens A, B, C, D, E (from shallow waters), to shadow tests; intervals of recovery from reaction indicated in seconds. The minus sign indicates failure to react. Temperature of

	•	Α	В	C	D	E		
1		6	7	7	8	6		
2		5 '	9	16	16	5		
3		10	10	15	15 (			
4		9	10	15	15	5		
5	• • • • • • • • • • • • • • • • • • • •	9 :	15 ,	15	25	6		
6		8	8 .	10	10			
7		- ;	- '	_		6		
8	*** **** ** ** ** * * * * * * * * * * *	10	10	10	,	60		
9		8	10	10	20	6		
10		8	9 <sup>t</sup>	10	10 1	5		
11		9 :	9	16	16	20		
12		- !		-				
13		8 1	8	10	10	6		
14. <b></b>		1	-	_ '	'	5		
15		13 :	, '	-	1	_		
16		15	15	15	` 15 <sup>†</sup>	12		
17		10	15	20	20 ∤	15		
18		10	12	12	_	12		
	;	i	,		1			

every physiological laboratory. The familiar "muscle-nerve" preparation affords a good example of such. Under proper artificial adjustment such type of vital machine can be made to react with some approximation toward the requirements of the mechanical theory. Other similarly devised examples are not unfamiliar, in which chemical processes take the place of the physical in the former.

7 | 12 | 15 | 20 8 | 12 | 12 | -

TABLE IV

Showing reactions of two specimens F and G, tested at 10 a.m. and 2 p.m., temperature 22° and 23° C.

,	F	G	F	G
	10 a.m.	22°	2 p. m.	23°
1,	40	360	15	20
<b>3</b>	30	60	10	30
3	30	180	12	45
<b>4</b>	33	60	10	30
5	60	50	18	45
6	45	50	15	50
7	100	90	12	35
8	_	60	13	80
9	300	35	1 - 1	100
10	40	120	15	40
n	30	300	12	110
12	180	60	45	90
13	43	90	75	15
14	180	105	20	90
15	75	90	20	90
16	75	130	12	105
17	470	150		80
8	50	150	15	85
19	60	225	11	45
20 <b>.</b>	150	90	18	_

#### TABLE V

Showing reactions of four specimens, F, G, I, J. In the first column is shown the reaction and recovery time of F and G at 3 p.m., temperature 25° C. In the second column is shown the reaction and recovery time of same specimens at 4 p.m. and with temperature at 20° C. In the third is shown the reaction and recovery times of the other two specimens under same conditions at 4:20 p.m., and at 21° C.

1	F	G	F	G	1	J	
1	18	13	60	60	18	20	
2	15	15	30	60	30	25	
3	14	15	40	50	25	25	
4	18	15	45	45	20	20	
5	11	. 9	45	35	10	16	
6	15	' 9	45	. •	7	20	
7	10	. 10	45		10	25	
8	10	10	40		20	25	
9	15	20	45	0	15	-	
o	10	10	45	-	. 15	-	

Now, how do the facts embodied in these tables compare with those exhibited by the methods just cited? Do the reaction phenomena conform in any fundamental respect with those of a muscle-nerve preparation, or with those induced in cardiac muscle by such chemical reagents as Ringer's solution? The briefest comparison of a record of one of the latter experiments with the tables under review will quite suffice to show that this is not the case. To further test the problem an attempt was made by my colleague, Dr. Rogers, to reduce the records to a curve by appropriately plotting them, and thus make the results more easily

TABLE VI

Showing comparison of reactions under shadow and tactile stimuli, as indicated by the reaction time given in seconds. Two specimens, A and B, were used, an individual record being made of each specimen, tested successively by each method.

ļ	Α			3
,	Shadow	Touch	Shadow	Touch
1	12	50	16	50
2	13	40	19	61
3	21	50	16	94
4	11	17	18	50
5	` 13	20	. 13	45
6	17	20	20	44
7	11	25	12	46
8	20	21	15	45
9	22	40	25	74
o	11	30	22	41

comparable. But this process only served to confirm the obvious figures of the tables, namely, that no comparison was possible. Thus the conclusion seems unavoidable that the foregoing verdict is beyond doubt.

But the query may be pertinent, ought the two series to be placed in comparison? To this may be answered, Why not? If the earlier assumption that such organisms are definitely adjusted machines be pertinent, and this has not been denied, indeed, is the very point at issue, then any burden of denial or proof must fall upon the defenders of the mechanical hypothesis. One further query remains, namely, may not these phenomena belong to that class of reactions known as physiological reflexes? Again the facts portrayed must be the sufficient reply, and that in the negative, I think. It would seem absolutely impossible to correlate such recovery times as ten seconds and three hundred seconds, or even many of greater extremes than that, at successive reactions under an identical stimulus, and with the briefest interval between.

#### EFFECTS OF CHANGED CONDITIONS

Attention has been directed in several places to the fact that specimens from various habitats exhibited certain indications of the fact in the varied types of behavior. In connection with these aspects it was suspected that probably other factors might be involved in the matter. Accordingly some attention was directed to this point, some few of the results being summarized in the following sections.

### Temperature

It was occasionally noticed that specimens showed a somewhat sluggish attitude of behavior at early morning, or when the temperature was below that of other periods. Accordingly a series of experiments was made to determine to what degree this might prove of consequence. First, specimens were placed in a vessel surrounded by ice, and the effects observed. It was found that as the temperature was reduced to about 15° C. the worms began to withdraw into the tubes and remained thus retracted during the continuance of temperatures of that of lower degrees, thus making impracticable any tests. Allowing the temperature to return toward the normal, about 20° C. or above, it was found that the specimens soon resumed normal relations, and reacted as usual. In the tables given there may be found a few of very numerous experiments made in endeavoring to gain some light on this feature of the problem.

It may be assumed, from analogous and long-established facts, that in these organisms there is a range of temperature, between what are designated as minimum and maximum, wherein the normal phenomena of behavior, as of other vital activities, have their average play. That either above or below these the phenomena tend to become exceptional or abnormal. This has been found to be true in the present instance.

While a comparison of the reactions of F and G as shown in Table V at temperatures of 25° and 20°, might strongly suggest an important effect due to that factor, on the other hand the reactions of I and J of same table at 21° does not seem to sustain that interpretation. Something of similar import is also shown in the records of the preceding table, where with but one degree of difference the difference in time reaction is very considerable. The same thing was shown from numerous other records not here given. Though the influence of temperature may not be negligible in relation to the problem it can hardly be claimed to be of primary or exclusive importance.

## Variation in Oxygen Content

In connection with the experiments relating to the influence of temperature on behavior it was thought that variation of the oxygen content of the water might have some effect. As a simple qualitative test it was attempted to vary the amount of free oxygen by the process of boiling a given quantity of sea-water for some fifteen or twenty minutes, then closing the flask with a rubber stopper, and after cooling to the normal temperature try the effect of immersing the specimens in the flask thus treated. This was done, two specimens being used, and the records of reaction tabulated. These failed to show in the slightest way any appreciable effect of the change. While the slightest variation of attitude of a given specimen could be critically observed, nothing could be seen to indicate the slightest signs of discomfort in any way, even after the specimens had been kept in the deoxygenated water for several hours.

The experiment was repeated on the following day, under the best precautions to guard against error. A Bunsen valve was used in the flask which permitted the egress of steam but automatically closed as soon as boiling ceased, thus precluding the ingress of air. The specimens were again immersed in the flask of boiled water,

properly cooled, and critically observed for several hours. Indeed they were kept in the boiled water for more than twenty-four hours, the flask all the while being closely stoppered with a rubber cork as before. A very few of numerous records are given in Table VII.

#### TABLE VII

In this table are given the reactions of two specimens, A and B, which had been placed in the deoxygenated water. As will be seen there is little or nothing distinctive in any way. Attention may be directed to the rather marked recovery time shown in A at the seventh and fifteenth test, and in B at the first. It was thought at first that A was likely to show the periodicity mentioned by Yerkes, hence the tests were carried on till this was clearly disproved, the same being evident in the reactions of B.

		A		ļ	В
•	Shadow	pa - man to	Shadow		Shadow
1	44 :	21	30	1	304
2	47	22	23	2	31
3	25	23	25	3	35
4	29	24	33	4	25
5	44	25	17	5	34
6	40	26	28	6	22
7	1530	27	27	7	33
8	39	28	17	8	22
9	58	29	16	9	27
<b>o</b>	24	30	19	10	37
1	33	31	27	11	50
2	35	32	24	13	38
3	38	33	33	13	43
4	39	34	43	14	55
5	945	35	37	15	42
6	36	36	30	16	33
7	28	37	21	17	41
8	30	38	35	18	32
9	24	39	70	19	37
10	26	40	41	20	43

Similar records of the same specimens were made in the usual aquarium water, or that obtained fresh from the harbor. But there is little to indicate any effect due to inadequate oxygen supply. After continuing in the boiled water for as much as forty-eight hours not the slightest indication of physical discomfort, or modification of behavior, could be detected.

As a modification of the experiment an attempt was made to displace the free oxygen by carbon dioxide. This was done in the usual manner by one of the assistants of the chemical laboratory. After boiling to drive off the free oxygen, the water was cooled and then charged with CO<sub>2</sub> from the generator and the flask closely stoppered. When an appropriate temperature had been obtained the same specimens were immersed in the carbonated water. For some time the specimens remained within the tubes. In two minutes one specimen protruded, but after some ten seconds retracted into the tube. In five minutes the other specimen did likewise, expanded the gills freely for ten seconds, then suddenly withdrew. This process was several times repeated by both specimens. Later they expanded very slowly, and when fully out the shadow test was applied but without the slightest This test was repeated several times and with exactly the same negative effect. After about half an hour the specimens had apparently become sluggish and showed signs of discomfort. By means of a glass rod which had been thrust through the stopper they were gently touched, but gave no response. This was variously repeated with the same results. Finally they were removed from the flask and placed in a dish of normal water, one specimen remaining protruded during the transfer.

In from fifteen to twenty minutes the specimens began to show signs of recovery, first to tactile stimulation, and inside of thirty minutes were also so far restored to normal activity as to react to the shadow test.

As in the former experiment with oxygen reduction these were repeated the following day and with essentially the same results. It would seem as if the immersion in the carbonated water had acted to induce anæsthesia, rendering the creature insensible to conditions. In no case was any injury perceptible, the specimens always recovering more or less readily when transferred to normal water. This in some cases took place after a prolonged immersion of more than an hour in the carbonated water.

This series of experiments were designed in the simplest way to afford some qualitative indication of the possible influences of a few of the most common and usual physical and chemical factors in matters of behavior. They are not of sufficient importance to warrant any explicit deductions, but are simply given as facts more or less directly related to phases of metabolism and behavior presumably more or less intimately correlated. While the effects of the supercharged water with CO<sub>2</sub> were not unexpected, those associated with the reduced oxygen content were something of a surprise. Both series of experiments would seem to suggest that certain organisms are capable of enduring inimical respiratory conditions which would be quickly fatal to others. They further suggest that the signs of distress sometimes observed in these creatures in the aquarium in which the water has become "bad" may not be due to lack of oxygen, not to the presence of excess of CO<sub>2</sub> but to toxic organic matters with which the water has become charged.

### MODIFIABILITY OF BEHAVIOR

While attention was directed to this feature in the earlier paper (pp. 304, 316), no special details were given. Early in July I obtained from Mr. Geo. Gray a colony of Hydroides which had been kept in his aquarium over the preceding winter. It seemed probable that some evidence might be obtained concerning the effects of long confinement under artificial conditions which might be of importance. The aquarium in which they had been kept was in the basement of the laboratory, and was but dimly lighted. It was, however, near one of the working tables about which there was more or less passing. A careful examination of the colony seemed to show that it was apparently normal, and in an average state of vigor so far as could be observed. On testing by the shadow stimulus it was found, however, that they were strangely negative, only a specimen here and there showing any response to the tests. The colony was taken to my private laboratory where they were placed in the most favorable conditions for accurate observation. Subjected here to the usual tests, at first not more than about 10 per cent responded in the usual manner. And after the first test or two of a series even this ratio decreased almost to the vanishing point. Two or three possible explanations may be suggested. First, that we have here a counterpart of the largely negative results found in specimens from deep waters, already referred to in an earlier connection, the dim light of the basement having operated as the similar dim light of the depths.

Second, the possibility that long-continued association of the presence of passing shadows unaccompanied by harm had induced a state of indifference to such stimuli, a not unsupported view of the case. Or, third, the possibility that long existence in the aquarium had acted to lessen the sensory activity, due in part perhaps to a lowered state of general vigor. Both my former discussion of this point, and the somewhat anomalous results obtained by Yerkes in certain cases, would seem to support the last alternative.

Whether one, or something of all these factors may share in the real explanation it would be of small consequence to discuss from so limited a body of facts. Some further hints may be afforded in the later accounts of these specimens. The colony was kept in the strong light on my laboratory table and tested daily for some two weeks, both by shadow and tactile stimuli. As a result of these it soon became apparent that a marked improvement was under way, and before the experiments were discontinued the several specimens were reacting up to about the average of normal specimens fresh from the sea. That we have here modified behavior growing out of environment, or experience, or both, seems more or less evident. Possibly the presence of strong light, associated with the tests referred to, had been operative in developing keener sensory powers. At any rate, the facts of modified behavior are beyond question. These serve to extend and confirm the experiments already mentioned, and warrant an assurance of a considerable degree of modifiability in these aspects of behavior upon the part of Hydroides.

### ASPECTS OF BEHAVIOR EXHIBITED BY THE TUBES

In the former paper (p. 313), a brief paragraph and a few illustrations were devoted to the problem of the significance of gravity as a factor in the behavior in these organisms. Additional facts

have since come to light which seem to have still more convincing significance, and a few further suggestions may be pertinent. Whatever of doubt may be involved in the observations and interpretations of the behavior of living organisms, whether in the aquarium or the native habitat, the aspects of behavior portrayed in the present case by the tubes themselves are beyond all dispute, at least as to matters of fact. In these structures there has been left a graphic and authentic record of behavior no less certain and convincing than that of fossils. Hydroides have literally lithographed a picture of behavior in minutest detail, a valid interpretation of which calls for no abstruse speculation nor the projection of novel or pretentious theories. These tubes are explicit expressions of the physiological and ecological activities of the creatures which build and occupy them, as certainly as are the structure and architecture of human habitations expressions of certain aspects of human life. Hence in these tubicolous structures one may trace the varying aspects of growth and behavior from week to week throughout the entire life history with a sense of assurance not easily disconcerted by the scare-crow of anthropomorphism.

There is no occasion for dogmatism on this point. One may not always be certain that a given interpretation may be beyond dispute, but as facts multiply involving data of habitat, distribution, experimentation, etc., doubts become less doubtful, and may give place to substantial certainty. Such is the case in the present instance. Facts have accumulated covering almost every phase of life history, from the emergence of the trochophore to the period of its attachment, and on through the whole of its varied life, both under artificial and natural conditions.

In the earlier paper attention was directed to Zeleny's observations as to the behavior of young worms at the time of attachment in relation to light and gravity. I have had recent opportunity to study the still earlier behavior of the trochophore itself, and also the aspects of the youngest tubes of the attached larvæ in natural conditions, which serve to still further confirm the former con-

<sup>7</sup> Biol. Bul., vol. viii, p. 309.

clusions. In common with many marine larvæ the trochophore of Hydroides when first emerging from the egg is positively phototropic, and also tends to swim near the surface. This soon changes and within about twenty-four hours it has become somewhat negative in relation to light, and still later becomes absolutely indifferent to this stimulus. Following up these facts it was found from an examination of a large number of colonies along the shore line that in many cases the larvæ had attached themselves under stones, in shaded places, in crevices, etc. On the other hand there were found many exceptions to this, especially from the deeper waters. For example, a large colony was obtained from New Bedford at a depth of two to three fathoms, attached to the sides, both inner and outer, of a granite-ware kettle. Had there been any definite compelling action of negative phototropism one should have found the larger portion of the tubes on the inner. because darker, side of the kettle. But the very opposite was the case, probably at least 75 per cent being on the outer and vertical sides of the vessel. On the assumption that attachment has some relation to food getting, or respiration, this is just what should have been expected, and this I am convinced is by far the most important factor in this phase of behavior. Colonies which settle in crevices, or secluded places soon come to show readjustments which have undoubtedly a definite relation to the above-mentioned ends. To attain these ends there will be, in many cases, just the complex and curious serpentine coilings which these tubes display.

The variable places and modes of habitat are of no small significance in relation to the problem of behavior here under discussion. In addition to features of this shown in the former paper still further facts have come to light. As intimated above habitat is extremely variable, and there is little to show that any single factor has a determining influence in the matter. While rocks, shells, etc., are the more common places of attachment, it is chiefly due to the fact that these are more abundant and available. They occur, not only on dead shells where they are extremely common, but are also found on shells occupied by living snails and bivalves. I have taken them on Sycotopus, Fulgur, Littor-

ina, Illyanassa, etc., and colonies occur also on the chelæ of the lobster, and doubtless many other such habitats. It can hardly be seriously contended that in such living habitats where they are constantly subject to change of position and relation any such factor as gravity or light has been dominant.

But one of the most interesting of habitats is that of the nets forming a part of a fish trap. It is of course well known that these traps are set up in water of from ten to twenty feet in depth, and are continued in use for some three or four months during the summer. In this particular case the tubes were taken from about the middle of the net, which would again preclude the direct action of either light or gravity in the matter of attachment. We have the further point of interest that some idea of the rate of growth may be inferred, since it must have taken place within the few months of a given season.

In this connection occasion must be taken to refer to a few features of behavior shown by Potamilla and Sabella. Since the observations noted in the former paper I have taken occasion to repeat and extend them. An examination of many specimens as they occur in nature failed to show any definite orientation to either light or gravity. As is well known these species form a tough, flexible tube, which may be easily bent in any direction. Loeb has found that Spirographis, an annelid having a similar tube, but growing to large size, shows a definite response to light. I have kept species of both Potamilla and Sabella in the aquarium for weeks, variously disposed as to light, but was not able to distinguish any definite evidence of such orientation.

#### CONCLUSIONS AND REFLECTIONS

On the basis of the so-called tropism theory, using this term in its current sense, these rather remarkable variations and individual differences of behavior are difficult to explain. If conditions of density, temperature, oxygen, etc., are to be regarded as primary determining factors, how with these more or less constant should behaviour differ; or with these differing to marked degree why should behavior continue more or less constant? Again,

why under conditions as nearly identical as they can be made should two, or any number of specimens react in such variable manner? According to the theory there should be the closest possible similarity. Yet this rarely if ever is the case; indeed, it is just the contrary, as the foregoing facts clearly show. In chemical and physical experiments the experimenter proceeds upon the assumption that given constancy of conditions he rightly anticipates constancy of results. Indeed, he may predict with mathematical certitude both the qualitative and quantitative character of the results. But this is just what the experimenter in problems of organic behavior can never predict, except in the most limited degree, and for but few organisms. And even then the results are far from certain exponents of this theory. To quote an expression from a well-known experimenter-"all the processes in our organism which can be explained on mechanical principles are as little phenomena of life as the movement of the leaves and branches on a tree when shaken by a storm" (Bunge).

On the other hand, if we may regard these organisms, not as mere machines, automata, but as individual beings endowed with an organization, both physical and physiological, capable of self-coördination and direction, whether from external or internal stimulation, or from pure spontaneity, then these variable phenomena of behavior are only such as conform to natural expectation. They form an integral part of that living world, from monad to man, whose correlated behavior, in its ultimate essence, differs relatively according to the complexity of the organism concerned.

In common with a growing body of students of behavior the writer is forced to regard the tropism explanation, whether applied to plant or animal, as but partial and superficial. The cases in which it has found its best illustrations have not stood the test of severe analysis. It is more than ever evident that, though we admit the intimate relation of chemical and physical processes, to every aspect of behavior, they are not of themselves explanatory except in the most partial way. Indeed it may well be questioned whether the all but universal disposition to explain the organic in terms of mechanics is not an actual reversal of

the natural sequence of relations. This has grown out of the assumption of the derivation of life from the non-living. Who shall say that the reverse may not have been the order of evolution, and that the inorganic may not be better interpreted in terms of organic life?

The writer has to confess more or less sympathy with a tendency to postulate the presence of certain psychic factors in the behavior of organisms. Notable among those who have recently advocated such factors may be mentioned Driesch, whose work is so well known as to call for no special citations. Under the terms "psychoid" and "entelechy" he has sought to attack the problem from a point of view which, though not distinctly new, has been given small consideration among the majority of experimental zoölogists. As one of the most distinguished of neo-vitalists his views are entitled to more than passing notice, though no attempt can be made in this connection to review them. Jennings, whose work on behavior has been so effective a check to dominant mechanical theories, fails to perceive any special merits in the methods and views of Driesch, designating his "entelechy" doctrine as a virtual abandonment of the problem. To this verdict I am unable to subscribe, believing that, whether or not the particular concept involved in "entelechy" prove of working value, as a postulate for a somewhat unusual mode of approach it is altogether scientific. While biologists may question the views of Preyer as to the nature and origin of life, or those of Helmholtz and Sir W. Thompson as to its mode of distribution, this is no warrant for discarding them as unscientific.

Psychic phenomena are just as real as any in nature, and just as much entitled to scientific consideration. Are they expressions of chemical or molecular stimuli alone, or are there involved other conditions of energy quite as distinct as either of the others? While an investigator here and there has ventured to suggest the crude hypothesis of a secretory function of the brain as adequate for the entire rôle of psychic activity, they have not included such acute students of physics and physiology as Tyndall and Huxley. As yet no careful investigator has cared to be sponsor for the application of mechanical principles in explanation of mental states.

And though one may not be able to demonstrate a distinctive kind of energy back of such phenomena, measurable by any known standard, this does not vitiate the essentially scientific nature of such a postulate as a working hypothesis. Indeed, in so far as the bevavior of man and higher vertebrates is concerned there has been small hestitation in assuming the operation of psychic factors. But who shall draw the line in the scale of life where such cease and where chemico-physical become dominant or absolute? Granted the existence and efficiency of psychic energy at any point in the field of behavior, why shall its rôle betabooed as in any sense an abandonment of the problem, or as disloyalty to the scientific method? It is not vital by what term we may choose to designate these extra-mechanical factors. Whether they be designated as "associative memory," "psychoid," "entelechy," "mnemism" or "physiological states," is of small consequence. But that there is back of such terminology subjective reality, of which behavior is the varied expression, is of profoundest import. And this, as I understand, is the fundamental contention of neovitalism as interpreted by Driesch.

The writer believes there is a growing conviction among many biologists that the declaration of Driesch that the "mechanical theory has failed all along the line," is not without strong support. In confirmation of this it may be pertinent to briefly refer to a few of the more recent and emphatic of these. Among them attention may be directed to the recent presidential address of Dr. Francis Darwin before the British Association for the Advancement of Science. In this address Dr. Darwin does not hesitate to predicate the operation of psychic factors in the behavior of plants, radical as this may be regarded by certain advocates of the mechanical view. Facing the age-long question as to the presence of consciousness in lower organisms, Darwin says: "It is impossible to know whether or not plants are conscious; but it is consistent with the doctrine of continuity that in all living things there is something psychic, and if we accept this view we must believe that in plants there exists a faint copy of what we know

<sup>8</sup> Science, September 18, 25, 1908.

as consciousness in ourselves." And in similar view Darwin discusses in considerable detail the problems of plant heredity, both as to ontogeny and phylogeny, under the so-called "Mnemic theory," a theory recently developed by Semon and Rignano, though earlier advocated in substance by Hering and Butler.

It is rather significant that at this same meeting, and before the Physiological Section of the Association, Dr. J. S. Haldane in his presidential address before the section assumed a similar attitude concerning fundamental problems of physiology. A single quotation may suffice to show the general attitude of this distinguished physiologist concerning the problems under review. "Now the first requisite of a working hypothesis is that it should work, and I have tried to point out that as a matter of fact the physico-chemical theory of life has not worked in the past and can never work. As soon as we pass beyond the most superficial details of physiological activity it becomes unsatisfactory; and it breaks down completely when applied to fundamental physiological problems, such as that of reproduction. Those who aim at physico-chemical explanations of life are simply running their heads at a stone wall, and can only expect sore heads as a consequence."

<sup>9</sup> Nature, October 1, 1908, p. 556.

## WOUND REPARATION AND POLARITY IN TENTA-CLES OF ACTINIANS<sup>1</sup>

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All animals, so far as experiments have gone, show capacity for prompt repair of damages. When an animal suffers an injury which is not necessarily fatal and which does not too seriously interfere with the normal life-processes, activities begin immediately in some or all of the tissues which share in the wound surface and these activities result quickly in a protective adjustment of some sort. If the wound is more than skin deep these activities appear to be primarily directed toward the restoration of a normal outside surface-normal, not necessarily as regards the form of it, but in the sense that the deep tissues which have been exposed by the wound are covered over by a tissue which is normally a superficial one. Subsequent to this immediate repair of the surface there may ensue more or less complete replacement of the lost deeper parts, with restoration of the original form. In a case like this we may distinguish two phases in the total process of reparation. (I use the word "reparation" in the every-day sense of making good an injury-by any means whatever, not in the restricted sense in which Driesch has proposed to use the word.) First there is a quick protective covering of deep tissues by a superficial tissue, then follows the slower regeneration of lost parts. It is with the first phase of the reparation—the process commonly spoken of as the closing of the wound—that I wish to deal in this paper. In a former paper (Rand '04) I have given some historical account of observations upon this matter.

The method by which a wound becomes closed depends more or less upon the degree of complexity of the organism and in some measure upon the size of the organism and the extent of the wound.

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The closing of the cut end of a stem of Tubularia, as described by Morgan ('01), is in some respects as simple a case as may be found. The cut end of a stem is closed in less than half an hour by means of a concentric centripetal extension of the old cœnosarc. This extension forms a thin plate transverse, or nearly so, to the axis of the stem. "This plate is composed of two layers of cells, of which there are a number of rows arranged concentrically between the center and the outer edge" (p. 69). It is to be inferred from the account that the two layers of cells represent the ectoderm and the entoderm. The final closing takes place at the center of this plate. The manner of formation of the plate, and the fact that the closing is complete are inconsistent with the idea that the process is one which depends upon the contraction of muscle fibers. Moreover, there are supposed to be no muscle fibers in the stem of Tubularia. Morgan's conclusion, that the closing is effected by activities of the comosarc cells akin to, if not identical with, amœboid motion, seems to be the most plausible view of the matter.

Experiments upon Hydra viridis afford similar phenomena. When the column is cut transversely the cut edges of the body wall slowly bend inward and within an hour the cut end is completely closed. The closed end is somewhat convex in form and both of the body layers are there present. It is perhaps possible that the closure in this case is accomplished by means of the muscle cells. Yet there is good ground for believing that activities of other kinds participate in the closing process even if they are not accountable for the whole of it. After the transection the cut edges at once bend inward slightly. This immediate change may possibly be due to contraction of the muscle processes. Again, if a column of Hydra is split lengthwise, each half column at once shows a tendency to roll into a cylinder with the ectoderm upon the outside, while at the same time it curls lengthwise into a flat spiral with the cut edges on the inside of the curve (Rand '99). This behavior, also, may result from muscular contraction. It should be noted, however, that the entoderm layer is much thicker than the ectoderm, and therefore the layer of muscle processes is not equidistant from the inner and outer surfaces of the body wall, but

considerably nearer the outer surface. Granting that ectoderm and entoderm are equally rigid layers, then a contraction of these muscle processes would bend the body wall so that the ectoderm would be everywhere on the concave side. The observed facts show just the opposite conditions—the ectoderm is always on the convex side. An explanation of these form changes on the ground of contraction of the muscle processes would necessarily involve the assumption that the ectoderm is more rigid than the entoderm, an assumption which may be regarded as consistent with the fact that the entoderm cells are highly vacuolated. But we may equally well abandon the muscle explanation entirely and suppose that the inbending of the cut edges and the spiral curling of the longitudinal half-column of Hydra are due to the existence of different degrees of tension in the two body layers. This explanation would, of course, apply also to the initial inbending of the cut edges of a transversely cut column. Loeb ('91) describes the inbending of cut edges in Cerianthus and suggests that it is due to unequal tension in the body layers. Child ('04a) gives an extended account of the behavior of cut edges in Cerianthus. He regards the initial inhending of cut edges as due to differences in the elasticity of the body layers, and points out his reasons for thinking that the mesoglea is the chief agent in causing the bending of the edges.

Some further considerations seem to me to argue against the view that muscle contraction is the only or chief factor in the closing of transversely cut edges of Hydra. Except for the initial inbending, which is somewhat abrupt, the process consists of a very slow and gradual extension of the body wall across the open end, and requires for its completion from fifteen minutes to an hour. The absolute motion here is incomparably slower than that involved in any of those ordinary activities of the normal polyp which we are safe in regarding as of muscular character. Again, the fact that the closing is complete offers difficulties to the muscle explanation. If muscular contraction plays a part in the process, it must be accompanied by a certain amount of rearrangement of the elements of the tissues, in order that the result may be a complete closing and that the two body-layers over the closed

end should retain uniformly their original thickness. Furthermore, if the cut end of the column of Hydra is crushed so that the wound is an extremely ragged one with partially detached fragments of ectoderm and entoderm lying together in a confused mass, there takes place a slow and orderly segregation of the material of the two body layers. Ectoderm flows into ectoderm, entoderm into entoderm; everywhere the entoderm retreats to a deep position while the ectoderm seeks a superficial one. The confusion of the two layers is corrected, the raggedness is smoothed over, and the result is a closed end with the two body-layers in normal relation to each other and of approximately normal thickness. Explanation of these activities by means of muscular contraction alone can hardly be imagined. Upon the other hand, there is every appearance of amæboid motion of the tissue elements.

Numerous experiments, such as those of Driesch ('95) upon the echinoderm blastula and gastrula, show that closing of wounds regularly takes place in embryonic tissues where muscle fibers or processes certainly do not exist. Driesch cut into fragments of various sizes the blastula and gastrula of Sphærechinus and Asterias. In fragments including one-half or more of the original forms the wounds promptly closed.

While the total evidence as to the mechanics of wound closing in Hydra is not conclusive, I am of the opinion that muscular contraction plays at most only a part in the process. The initial abrupt inbending of the cut edges is doubtless due either to muscle contraction or to some preëxistent difference in the elasticity or tension of the body layers, but in the complete closing of the wound a very important rôle, if not a leading one, is taken by something akin to amœboid cell motion.

In amphibian larvæ, as shown by Born ('96), extensive losses of epidermis are repaired in the course of an hour by means of a concentric advance of the epidermis from all sides toward the center of the wound, and without increase in the number of cells. There are no muscle fibers concerned in this process. Born found no evidence of amæboid migration of individual cells. He regarded the advance of the epidermis as resulting from a state of

tension in the epidermal sheet, this tension being due to a tendency of each individual cell to flatten itself so as to cover a maximum surface.

In the earthworm (Rand '04) the closing of a wound involves (These statements refer to the healing of an anterior cut end. Conditions at a posterior cut end are in certain respects different from those at an anterior cut end, as I shall show in a paper now in preparation.) First, immediately after transection of the body, contraction of the muscle layers in the vicinity of the wound ensues, whereby the wound surface is considerably diminished in area. In the second place, a cicatricial plug is formed, closing the exposed coelomic cavity. The material of this plug appears to be chiefly leucocytes, which are probably transported to the region of the wound by means of the extensive forward flow of body fluid which occurs immediately after the cutting. Then, thirdly, the epidermis advances from the region of its cut edges across the surface of the cicatrix so as either to cover over the cut end completely, or else to establish connection with the epithelial layer of the digestive tube. During this advance of the epidermal layer the cells collectively always present to the exterior a smooth and continuous surface, yet the movement of the layer as a whole is distinctly conditioned by the independent migratory movement of the individual epidermal cell. No cell-division takes place during this process.

In the stem of Tubularia and in Hydra we have a relatively simple kind of organization and transection produces a wound of small area as regards both cut tissue and exposed cavity. In the earthworm the organization is of much greater complexity and transection results in a relatively large wound involving many kinds of cut tissues and the exposure of large cavities. Under the former conditions the method of wound closing is a comparatively simple one; under the latter conditions it is more complex. What, then, we may inquire, would be the manner of reaction to transection in the case of a tubular structure possessing the simpler organization of Hydra, but whose dimensions are more nearly like those of the earthworm? The possibility of obtaining an answer to this question was suggested to me by the luxuriant size of certain

actinians which occur about the Bermuda Islands. The many crevices and miniature caverns in the soft calcareous rock between tide levels house myriads of beautifully colored anemones. Of various sizes, some of the larger of them, when seen expanded in the water, appear as big as a man's head, and the extended tentacles have in general the dimensions of good sized earthworms. In one of these large tentacles we have essentially the same structure as in Hydra—the typical cœlenterate condition. But the gastro-vascular cavity has enormously greater cross-section both absolutely and relative to the thickness of its wall. Further, there is much higher differentiation of muscle fibers.

The experiments which I am about to describe were carried out at the Bermuda Biological Station for Research in the summer of 1907. Some supplementary observations were made at the same place in the summer of 1908. The subject of most of the experiments was Condylactis passiflora (for a description of the species see McMurrich, '89), the largest and most gorgeously colored of the Bermuda actinians. Most of the experiments were repeated upon a member of the genus Aiptasia. I was unable to identify the species to a certainty, although it agreed fairly well with McMurrich's description of Aiptasia annulata. Condylactis occurs in great abundance about Agar's Island, upon which the Biological Station is situated. Comparatively small individuals were selected for experiment, as being more conveniently maintained in healthy condition in small aquaria. Individuals under experiment were kept in large glass vessels of sea water, which was changed twice daily. They were fed small pieces of raw fish, usually once a day. Treated in this way they remained in good condition, so far as I could judge, for ten days—the longest period during which any one individual was kept under observation.

## DESCRIPTION OF EXPERIMENTS

An individual of Condylactis which furnished tentacles for several experiments had the following dimensions when well extended.—Height of column, 75 mm.; diameter of disc, 60 mm.; length of the larger tentacles, 70 to 80 mm.; diameter of these

tentacles at base, 10 or 11 mm.; diameter near tip, nearly 4 mm. The following experiment was repeated some twenty-five times with uniform results.

When the actinian was well expanded, a pair of sharp scissors was cautiously brought near a tentacle without contact with any other tentacle, and by a single quick clip the tentacle was severed about midway of its length. The behavior of the excised fragment will be considered later. Immediately after the cutting, the stump of the tentacle collapsed and contracted down close to the disc. Not quite simultaneously with this contraction of the injured tentacle, but closely following it, several of the tentacles nearest the cut one contracted more or less and in such a way as to bend in over the cut tentacle, while at the same time the region of column and disc bearing the contracted tentacles became deeply invaginated. This invagination took place so that the cut tentacle was at its deepest part. As a result of all this contraction, the injured tentacle was completely engulfed by surrounding parts, for the neighboring tentacles curved in over the invaginated region so that the cut one was deeply hidden from view. I did not attempt to prevent this contraction for, even if it had been possible to prevent it, I preferred not to introduce into the conditions affecting the animal any disturbing influences beyond the operation itself. After a period of time which varied greatly in different experiments, usually amounting to five or ten minutes when the cut was made about midway of the length of the tentacle, the contracted region of the animal began to relax and expand. When the expansion had progressed far enough to bring the cut tentacle into view, it was almost invariably found to present the conditions represented in Fig. 1 (Plate I). The stump of the injured tentacle was somewhat distended and its cut end appeared closed. This closed end was hemispherical except for the presence, at its center, of a small cylindrical projection. The expansion of the contracted region having once begun, progressed rapidly until, usually within fifteen minutes after cutting, all the uninjured tentacles were fully extended, while the stump of the injured tentacle had extended to nearly its original dimensions. Fig. 2 represents the condition of two cut tentacles as they appeared

after having regained full extension. The diameter of the distal portion of the stump in many cases seemed markedly greater than the diameter of the corresponding region of the tentacle before it was cut, while the length of the extended stump was usually slightly less than the length of the corresponding portion of the uninjured tentacle. In full extension the distal end of the stump retained the hemispherical form and the projecting cylinder which we may conveniently designate as the nipple.

The color and general appearance of the tissue at the closed end of the stump deserve notice. The outer surface of a normal extended tentacle is marked by very narrow and somewhat irregular transverse bands. These bands are represented on the proximal portion of the normal tentacle on the right side of Fig. 2. The bands are whitish and opaque, and appear to be made up of more or less blended spots. Between these bands the tissue is of yellowish brown color and very translucent. When the tentacle contracts the darker colored translucent zones appear to contract more, and as a consequence the whitish parts are so crowded together that the bands become less conspicuous or even quite indistinguishable to the eye. Therefore the contracted tentacle appears lighter colored and more opaque than the extended tentacle. The nipple upon the closed stump is whitish and densely opaque. The surface of the hemispherical wall which closes the end of the stump exhibits a gradation from whitish opacity to yellowish translucency. Immediately around the base of the nipple the tissue is whitish and opaque like that of the nipple itself, while, proceeding toward the equator of the hemispherical end, these two qualities gradually shade off into the yellowishness and translucency, respectively, of the lateral wall of the tentacle. (In the figures the opacity of the nipple and the region surrounding it is indicated by the dark shading.)

The cut end of the stump is functionally closed. In the normal tentacle there is a certain amount of internal fluid pressure. By this gentle pressure the extended tentacle is kept full and plump, and the act of extending after a contraction is doubtless facilitated. When a tentacle is cut, so that the internal pressure is released, there follows instantly a collapse of the walls, which is

entirely independent of any contraction of the tissues themselves. At the center of the tip of a normal tentacle there is a minute pore. Presumably this pore is kept closed while the tentacle is extended. If, however, the animal is suddenly lifted out of the water, fine jets of fluid spurt with considerable force from the tips of the tentacles. It seems to me probable that when a tentacle contracts this terminal pore is opened so as to allow escape of internal fluidan arrangement which would, apparently, facilitate quick contraction. However, I have no experimental proof of this matter. When the stump of a cut tentacle has become extended after the fashion described, the cut end is closed in the sense that it is able to resist the pressure within the tentacle, so that the fluid does not escape and the tentacle remains well distended. But this closure of the cut end is not necessarily permanent. Usually, when the stump contracted, the nipple persisted during the contraction phase. However, it was several times clearly seen that, in the contracted condition, the axis of the nipple was pierced by a fairly In occasional cases of extreme contraction, the large pore. nipple disappeared and the end of the contracted stump became again broadly open. In such cases, at the beginning of the process of extension the opening was rapidly narrowed and the nipple reappeared.

The foregoing account refers to tentacles which were cut midway of their length. Tentacles cut nearer the base or nearer the tip differed in some points of behavior from those which were cut midway of their length. When a tentacle is transected near its base, the resulting contractions are much more marked and persist for a longer time. The invagination of disc and column into which the cut tentacle is withdrawn is deeper than when the cut is midway of the tentacle's length, and from a half hour to an hour may elapse before sufficient relaxation takes place to bring the cut tentacle again into view. The closing of the cut end and the formation of the nipple occur as already described. The nearer the tip the tentacle is cut, the less is the amount of the resulting contraction and the shorter is the duration of it. If only the distal fourth or fifth of the tentacle is cut away the contraction may not extend beyond the stump of the tentacle itself. For example, a piece one or two

millimeters long was clipped from the end of each of four of the smallest tentacles of a small individual of Condylactis. The four tentacles contracted down close to the disc, but there was no marked contraction of the disc itself, or of other tentacles. than one minute after the cutting the cut ends were closed, the characteristic nipples had appeared, and all four tentacles were in process of extension. Pieces of similar size were then clipped from the tips of four of the largest and most fully extended tentacles of the same animal. These larger tentacles contracted to about one-half their former length-no more. The closing of the cut ends and formation of nipples were accomplished within four or five seconds and the tentacles were again fully extended within one minute. Under these circumstances one has free opportunity to see all that can be seen of the process of closing. Only a brief instant elapses before the cut edges begin to curve inward so as to diminish the size of the opening. Then, as the tentacle begins to extend, the nipple is formed by a gradual tightening of the wall around the opening. This experiment was repeated upon eleven tentacles of other individuals. In all cases the tentacle shortened almost to the disc. This contraction is not instantaneous, but occupies one or two seconds of time. In four cases the nipple was formed during the contraction and the tentacle then immediately began to extend with the end already closed by a strongly developed nipple. A distinct nipple was formed in every one of these eleven cases.

In one experiment, where the distal third of a tentacle was cut away, the contraction of neighboring parts was so much less than usual that the cut tentacle remained in view, so that I was able to watch the closing process from the beginning. After the cutting the tentacle instantly collapsed, and more slowly contracted close to the disc. Immediately after this contraction the opening at the cut end of the stump was quite irregular in outline—not circular—and in area not noticeably larger or smaller than it was at the instant before contraction took place, but its edges were everywhere slightly bent inward. Within the second minute after the operation the area of the opening began to diminish rapidly. At the end of three minutes the opening was markedly smaller and

its former irregular outline had been smoothed down to an approximately circular one. Then the stump began very gradually to extend, the continued diminishing of the opening being more marked as the tentacle expanded. Very soon after the tentacle began to extend, the nipple-like condition at the cut end became evident. It appeared as if a ring of material around the opening contracted more and more sharply, while all the rest of the tentacle wall was relaxed and distended. After the nipple had become well formed, the tentacle rapidly expanded and at the end of ten minutes the stump had regained practically its original dimensions, presenting then the appearance already described and represented in Fig. 2.

In many cases where only a short piece was cut from the tip, the injured tentacle was thrust into the mouth and retained there for a quarter of an hour or more. In one series of eleven experiments, this behavior was observed in six cases. The act of inserting the tentacle into the mouth was performed as the tentacle extended, after the usual contraction which followed the cutting. During the process of extension the tentacle, already closed and bearing a nipple, was bent in toward the mouth opening and slowly pushed into it, while its entrance was assisted by characteristic swallowing motions of the gullet. In this way one-half or more of the length of the tentacle was engulfed. The walls of the tentacle were pressed tightly together by the lips of the gullet while the unswallowed part of the tentacle remained fully expanded and much distended by internal pressure. If I gently pulled the tentacle out of the mouth, it was usually reinserted. One cut tentacle which had been repeatedly withdrawn from the mouth was found reinserted nearly an hour after the cutting. In one case the stump of a tentacle whose distal half had been cut away was put into the mouth, but I did not observe this behavior in any instance where more than half the tentacle had been removed. This act took place with such promptness and precision, when it took place at all, that I cannot regard it as due to any accidental contact of tentacle and mouth.

The closing of the cut end of a tentacle having been accomplished, the stump thereafter behaved as nearly as possible like

a normal tentacle. It remained extended while the other tentacles were extended and participated in any general contraction of tentacles, as well as responding in the usual way to individual tactile stimulation.

Experiments similar to those which I have described thus far were performed upon more than fifty tentacles and with fairly uniform results. What, now, is the further history of the closed end of the cut tentacle?

Usually on the day following the operation it was noted that the nipple was distinctly smaller than at first. This diminution in the size of the nipple progressed gradually and on the second day, in the majority of cases, the nipple had entirely disappeared. The closed end of the tentacle was then smoothly hemispherical. The point at which the nipple had disappeared was marked by a small whitish spot. During the period of retrogression of the nipple the whitishness and opacity of the hemispherical wall of the closed end gradually gave way to yellowishness and translucency. No pore could be found in any case at the point where the nipple had disappeared. Even the whitish spot which at first marked this point gradually faded away in the next few days.

Within the longest period that any one tentacle was kept under observation—about seven days—there were no signs of regenerative activity at the closed cut end nor was there any very conspicuous regulatory change in the proportions of the tentacle. In some cases it seemed to me that the repaired stump was longer than at first and that its distal end had become somewhat more tapering, like that of a normal tentacle. But the change, if any, was very slight and I undertook no measurements.

Experiments similar to those which have been described were made also upon specimens of Aiptasia whose dimensions were approximately as follows.—Height of column, 45 mm.; diameter of disc, 40 mm.; length of tentacles, 50 to 55 mm.; diameter of largest tentacles, 2.5 to 3 mm. These tentacles were of nearly uniform diameter from base to tip instead of tapering as in Condylactis. As these dimensions show, this actinian was much smaller than the individuals of Condylactis which were used. The tentacles of Aiptasia have extremely thin filmy walls, which are almost

perfectly transparent except for irregular transverse bands, which are whitish and opaque.

The results of transection of tentacles in Aiptasia were substantially the same as in Condylactis. The following differences as to details may be noted. The contractions caused by the cutting were less extensive and of shorter duration than in Condylactis. Even when the tentacle was cut near its base, so that more or less contraction of neighboring parts of disc and column and of the nearby tentacles ensued, there was full expansion of these parts within two to five minutes after the operation and the stump of the cut tentacle extended to its full length with a conspicuous nipple on its hemispherical closed end. The nipples were, upon the average, longer in proportion to their diameter-more slender --than in Condylactis. The nipple was whitish and opaque, while the wall of the closed end showed a gradual shading-off from the whitish opacity immediately around the base of the nipple to the transparency of the lateral wall. When only a very short piece was clipped from the end of a well extended tentacle of Aiptasia there was only a slight contraction of the tentacle, and the closing of the cut end and the formation of the nipple took place almost instantaneously. In one case a large tentacle of the innermost cycle was transected very near its base. Fig. 3 shows the appearance of the stump of this tentacle, a half hour after the operation, as seen in a lateral view of the upper end of the column. This case showed more conspicuously than any other the important part played by the nipple in the closing of the wound. Here a large portion of what remains of the wall of the base of the tentacle is gathered in to form the nipple and consequently the extreme lower part of the wall of the tentacle is pulled down so as to lie almost in the plane of the disc, being only slightly convex upward. The nipple has every appearance of a strong sphincter operating to resist the pressure of internal fluid.

In a few exceptional cases, both in Condylactis and in Aiptasia, the cut end of a tentacle closed without the formation of a nipple. The tissue at the center of the closed end appeared merely somewhat thickened.

A modification of these experiments consisted in making an

incision into the side wall of a tentacle without completely severing any part of it. With the tips of the scissors a cut was made extending one-fourth or one-third the distance around the circumference of a large tentacle of Condylactis in a plane transverse to the axis of the tentacle and usually somewhere near the middle of its length. Tentacles injured in this way collapsed and contracted in the usual manner. After a period which varied from a few minutes to the greater part of an hour the tentacle again became extended and was then found to be sharply bent at the region of injury, the cut place being on the concave side of the bend. The bend was so abrupt that the injury itself was completely hidden from view. I found it possible, however, by very gentle manipulation of the tentacle, to unbend it enough so that I could see the wounded place. By this means I was able to determine that the original slit had been contracted to a small circular pore. In the persistent sharply bent condition of the tentacle there was probably no open passage through this pore. The tissue immediately around the edge of the pore was white and very opaque, these qualities shading off gradually into the yellowish translucency of the tissue more remote from the injury. This whitish zone corresponded in extent to the contracted region of the tentacle at the bend. Furthermore, the degree of whitishness and opacity was in proportion to the amount of contraction. The injury lay at the exact center of the zone of contraction, which was responsible for the bending of the tentacle. The conditions which have just been described persisted for two or three days, but the size of the pore diminished all the time. Fig. 4 shows the appearance of a tentacle three days after an injury of this kind was inflicted. The pore, which could be seen by gently straightening out the bend, was not larger than the point of a common pin. After another day or two (four or five in all) the pore was completely closed and the tentacle began to straighten out. The unbending is very gradual, requiring several days. The whitish appearance at the concave surface of the bend persists so long as the bend is conspicuous, and gradually fades away as the tentacle straightens out. But even after the tentacle is well unbent a small white spot marks for a time the point where the pore closed.

This experiment was made upon five tentacles, with similar results in all cases.

This behavior of cut tentacles constitutes a very definite reaction to wounding and one which must have a certain protective value to the entire organism. Is this reaction purely a local matter—is it effected exclusively by the tissue which bends in to close the cut end and form the nipple, or, upon the contrary, is it dependent in some way upon the organism as a whole? What will happen at the distal cut end of a tentacle which has previously been severed from the column?

Experiments similar to the one about to be described were repeated many times and with very uniform results. The case described in detail is a typical one. A large well extended tentacle of Condylactis was selected and by means of a single quick clip of the scissors it was severed, near its base, from the column of the actinian. As it fell to the bottom of the aquarium the detached tentacle collapsed, owing to the release of the internal pressure, and contracted to about one-half its original length and diameter. At the proximal cut end of the severed tentacle the walls almost immediately bent inward slightly so as to diminish somewhat the size of the opening and this inbending soon became marked by much wrinkling and puckering, being in that respect very unlike the closing of the wall as observed at the distal cut end of an attached stump of tentacle. The behavior of a proximal cut end will, however, be considered further in another connection and now we are especially concerned with the comparison of the behavior of distal cut ends of attached and detached tentacles. As soon as possible after the detached tentacle had settled upon the bottom of the aquarium it was cut transversely and about midway of its length. The distal portion showed only slight additional contraction in response to this second cut and remained motionless on the floor of the aquarium. The proximal piece, however, contracted until its length was no greater than its diameter. Further, it became very much wrinkled and distorted, and in this shapeless condition it writhed and contorted itself almost continuously for a half minute, then gradually became quiet and

assumed a more smoothly cylindrical form. The distal opening of the proximal piece, as well as the proximal opening of the distal piece, was at first slit-like in form and remained so for several minutes, apparently as a persistent result of the pressing together of opposite edges of the wall in the act of cutting. The cut edges were, from the first, slightly bent inward.

In some preliminary experiments with detached tentacles I had found that, when a tentacle lay upon the bottom of the aquarium in a collapsed and contracted condition, a very slight current of water directed by means of a pipette into the open end of it often resulted in a partial extension and inflation of the tentacle, and this effect might endure for several seconds. It often appeared as if the expansion were due not entirely to the direct mechanical effects of the slight pressure, but, at least in part to a relaxation of the tissues of the walls, induced perhaps by the stimulus of the pressure. In the present experiment, about eight minutes after the second cutting I directed a gentle current of water from a pipette against the proximal opening of the proximal piece with the result that the piece became plump and smooth and extended noticeably in length. In this slightly extended condition it became apparent that there was a definite circular region of contraction around the distal opening which then appeared as a round pore. In a few seconds the piece contracted again. In so doing, the distal hole enlarged markedly, as if by a relaxing of circular fibers, and, losing its circular outline, became irregular with some approach toward its previous slit-like form. Sixteen minutes after the second cutting this injecting operation was repeated. This time the result of the slight internal pressure was a very marked expansion in diameter with only slight change in length. The piece swelled out to a bulb-shaped form, while its distal region, sharply contracted, formed the characteristic cylindrical nipple traversed by a small circular opening. Fig. 5 shows the appearance of the piece at this moment of expansion. The proximal cut end, although it shows a certain amount of contraction, is yet wide open.

I then turned my attention to the distal portion of the severed tentacle. Twenty minutes after the original operation I cut a

small fragment from its distal tip. Within two minutes the distal cut end had closed in, and a distinct nipple had formed in substantially the same manner as when a small fragment is removed from the tip of an attached tentacle. The appearance of the piece one hour later is shown in Fig. 6b. The conspicuous nipple is still present. The proximal end is broadly open and deeply folded.

The appearance of the proximal portion of the severed tentacle at this same time—about one hour and twenty minutes after the original operation—is shown in Fig. 6a. The tentacle was not artificially inflated this time, but was drawn just as it was found without disturbance of any kind. It has the same general form as that shown in Fig. 5. The nipple is narrower than before. There is a distinct whitish zone of contraction around the base of the nipple. The proximal end is broadly open and its inbent walls are much folded and wrinkled. Twenty-four hours after the beginning of the experiment the two pieces, still distinctly alive, presented much the same conditions as those represented in Figs. 6a and 6b. The nipples, however, were much less conspicuous and no visible pore could be detected at the distal end of either piece. The proximal piece was fixed in mercuric chlorid and a drawing of its proximal end was afterwards made (Fig.7).

The need of a little internal pressure to induce the expansion of the piece of severed tentacle led to the arrangement of a simple apparatus whereby such pressure could be applied and controlled in a more practicable way than by means of a pipette. A short piece of glass tubing was drawn out to a curved and tapering point. To the other end of the piece of glass tubing a small glass funnel was connected by means of a bit of rubber tubing. The funnel was supported from a wooden standard and the drawn end of the glass tubing was allowed to dip into the water contained in a small glass bowl. A large tentacle of Condylactis was detached from the column and removed to this small vessel. By use of fine forceps its proximal cut end was drawn over the tip of the glass tube and tied onto it by means of a ligature of soft thread. The stimulus of cutting followed by the irritation due to picking up with forceps and to tying the ligature all combined to induce con-

siderable contraction of the tentacle. But, so long as irritation of the distal end of the tentacle was avoided, the contraction did not exceed two-thirds of the original dimensions. A few minutes after it had been tied onto the tube the tentacle began to make spontaneous movements, alternately extending slightly and then contracting again. It also bent or waved from side to side somewhat as normal tentacles often do. These movements, however, involved only comparatively slight changes in the degree of contraction. The tentacle, under these conditions, never fully extended to its original dimensions. Then water was dropped into the funnel until it stood in the tube at a height of about twenty-five millimeters above the surface of the water in the bowl. Under this internal pressure the tentacle swelled out somewhat and assumed the general form of a normal tentacle, but its dimensions remained about one-half its original dimensions when extended. Further, the spontaneous movements ceased and the tentacle became perfectly rigid. It gave not the slightest response to tactile stimulation. If struck violently and pushed from side to side it merely sprang back to its original position just as any inanimate thing of like form and texture, and similarly under internal pressure, would have done. The pressure was increased up to thirty-five millimeters without in any way changing the behavior of the tentacle. (In another case, the tentacle contracted under twenty-five millimeters pressure, raising the column of water in the tube.) At this point in the experiment the distal half of the tentacle was clipped off. Instantly the proximal half collapsed and contracted close to the end of the tube. After the lapse of about half an hour the contracted stump of tentacle occasionally showed 4 a slight tendency to extend. Thereupon, at one of these moments of extension, a gentle internal pressure was produced by raising the water in the tube to a height of 10 mm. The piece of tentacle at once swelled out conspicuously and attained approximately the dimensions which it had before the distal half was cut away. But, when thus expanded, its recently cut distal end was found to be practically closed. The appearance of a tentacle under these conditions is represented in Fig. 8. At the tapering tip of the tentacle appeared to be a small circular pore. Yet this pore must

have been virtually closed, for there was no obvious leakage of water from the tube. The pressure was increased to 25 mm. and still there was no escape of water at the tip of the piece of tentacle. In this particular case the cut end did not assume conspicuously the nipple form, but in other cases, as will be shown below, it did. After maintaining itself in this condition for some ten seconds the tentacle abruptly shortened and at the same instant the distal pore enlarged to a wide irregular hole, as if, in the act of shortening, circular fibers guarding the practically closed distal pore had suddenly relaxed. The behavior just described was observed repeatedly in the same stump of tentacle. Under gentle internal pressure, applied at an instant of tendency toward expansion, a certain amount of extension resulted, during which the cut distal end assumed a tapering form and the passage through its tip was closed tightly enough to resist the internal pressure. These conditions having obtained for a few seconds, then the tentacle would shorten and collapse, and in so doing the relaxing of the contraction at its distal end allowed the distal pore to become broadly open. In this experiment the piece of tentacle was left attached to the glass tube over night. Next morning the tentacle was found lying upon the bottom of the vessel, having broken away from the tube. Fragments of the tissue still clung to the ligature. (In other similar experiments it was found impossible to secure the tentacle onto the tube for more than a few hours. The detaching of the tentacle was doubtless due to the pressure of the thread upon the soft and delicate tissues.) The piece of tentacle was in a slightly extended condition. Its distal end tapered to a point and no opening through it could be detected. The proximal end was wide open and its edges, ragged in outline, were rather sharply bent inward. On the following day, the second after the operation, the tentacle was still alive, but showed signs of degeneration. When contracted (that is, shortened) to its utmost I discovered a minute circular pore at the center of the distal end, but when the tentacle was extended (elongated) this pore become invisible.

Experiments similar to the one just described were repeated several times upon tentacles of Condylactis with fairly uniform results. Although in some cases the form of the distal tip in

extension resembled that shown in Fig. 8, usually a more or less definite nipple was present. Fig. 9 shows the appearance of a piece of tentacle ligated onto the hydrostatic tube and slightly distended under gentle pressure. A nipple, although a short one, is distinctly present. This condition appeared thirteen minutes after the distal portion of the tentacle had been cut away. In this experiment the behavior of the ligated proximal piece of tentacle, as well as that of the distal piece which had been cut away from it, was watched closely and constantly for forty-five minutes. Within three minutes after the cutting away of the distal piece the hole in the distal end of the ligated piece had contracted noticeably in area and its edges, at first somewhat angular, had become smoothly circular. As the piece alternately shortened and elongated the size of the hole varied considerably, usually being larger when the tentacle was shortened. Its average size diminished steadily but very slowly. One hour after the beginning of the experiment the tentacle appeared as in Fig. 10. (The figure shows an oblique end view of the fragment.) In this slightly elongated condition of the tentacle the opening was a small circular pore. A whitish zone around the pore indicated a state of contraction in the tissues of that region. Four and a half hours after then, beginning of the experiment the pore was still smaller, being quite, invisible at times. In this experiment, and commonly in the others the ligated piece usually extended in length only, remaining very small in diameter as if under persistent contraction of circular fibers. However, during the fifth hour of the experiment the tentacle was twice seen to swell out, apparently by a relaxation of circular fibers, but in so doing the contraction at the distal tip. persisted and the pore became no larger. It should be borne in mind that in all of these experiments with the pressure tube a little internal pressure was applied whenever a tendency to extend was noted, for by that means a much clearer demonstration of the condition of contraction at the distal end was obtained.

If fragments of tentacles were allowed merely to lie upon the floor of the aquarium for twenty-four hours after excision the distal cut ends at the expiration of that time were smoothly tapering in form, nearly or quite closed, and in the habitually much contracted condition of such fragments and in absence of internal pressure, there was usually little suggestion of the nipple form at the distal tip.

This same experiment was performed upon tentacles of Aiptasia. The following case is a typical one. A large tentacle was excised near its base and ligated onto the hydrostatic tube. A sharp contraction took place, the tentacle being reduced to about one-fourth its original extended dimensions, both as to length and diameter. After fifteen minutes, during which the tentacle remained in this contracted condition, internal pressure was applied. Under the influence of 30 mm. water pressure the contraction was partially overcome and the tentacle swelled to about twice the length and diameter which it possessed just before the application of the pressure, that is, to about one-half its dimensions before excision. This expansion appeared to be perfectly passive on the part of the tentacle-rather a direct mechanical effect of the internal pressure than a result of any spontaneous extension of the tissues themselves. While in this stretched condition, about two-thirds of the distal end of the tentacle was quickly clipped off. Instantly-in the fraction of a second, and before the water in the glass tube could fall more than some 8 mm.—the cut distal end of the stump of tentacle closed and a prominent nipple was formed. tentacle as it appeared at this moment is shown in Fig. 11 (Plate 2). Having remained in this condition for several seconds, the tentacle then shortened abruptly, the nipple disappeared, and the distal end became broadly open, allowing the water column to fall to zero. At frequent intervals thereafter tendency to extend was noted and at such moments water was introduced into the tube. Sometimes no marked extension followed and the water merely ran through the tentacle and escaped at the open cut end. But, four times within ten minutes, extension accompanied by closing of the cut end and formation of the nipple did take place and the conditions of Fig. 11 were repeated. Each time the nipple sustained a pressure of some 30 mm. of water with only slight leakage, or none at all.

To demonstrate the closing of the cut distal end and the formation of the nipple in excised tentacles of Aiptasia the use of the hydrostatic apparatus was essential. When a piece of tentacle, cut at both ends, was allowed merely to lie upon the bottom of the aquarium, it remained in a strongly contracted condition for hours—so contracted that water could not be injected at the proximal end by means of a pipette as was done for Condylactis. From the instant of cutting the distal end was virtually closed owing to this condition of general contraction.

The behavior of pieces of Condylactis tentacles and pieces of Aiptasia tentacles under the influence of artificial internal pressure is substantially the same. Such differences as appeared are parallel with differences noted in the behavior of tentacle stumps left attached to the columns in the two species. Whether attached naturally to the column or artificially to the hydrostatic tube, the distal cut end of an Aiptasia tentacle closes and forms the nipple much more promptly and decisively than does the Condylactis tentacle under similar circumstances. Further, the nipple is a slightly more conspicuous and constant feature in Aiptasia than in Condylactis.

A fairly definite difference was observed in the behavior of those pieces of Condylactis tentacles which were attached to the hydrostatic tube and those which were allowed to lie upon the bottom of the aquarium. Under the latter condition contraction in length was the conspicuous feature and when the contracted tentacle relaxed somewhat there was commonly only a moderate increase in length, while the diameter remained large (Figs. 13–15). In the case of a piece ligated onto the tube, contraction in diameter was more marked and when the piece extended it was usually an extension chiefly in length, the diameter remaining much contracted except as it was increased mechanically by internal water pressure (Fig. 9).

### DISCUSSION AND SUMMARY

What is the means by which this prompt and characteristic closing of a distal cut end of a tentacle is effected? There can be hardly any doubt that the initial closing, which is usually accompanied by the formation of a nipple, is chiefly dependent upon contraction of muscle fibers. The following facts point toward

this conclusion. In the first place, the closing reaction may follow the cutting almost instantaneously. There is, indeed, considerable variation in the promptness of the reaction depending mainly upon the level at which the tentacle is cut. It is when the transection is located at the tip of the tentacle that the reaction is most prompt. Here the closing takes place so quickly that the internal pressure is released only for an instant. The tentacle does not have time to collapse. A cut at the extreme tip often causes comparatively little contraction of the tissues of the tentacle—particularly in Aiptasia. Therefore the original conditions of extension and internal pressure are restored immediately after the cutting, or, in fact, they are scarcely interrupted by the cutting. But when the transection is nearer the base, then there is much contraction of the stump of the tentacle and several minutes may elapse before its cut end is closed. However, during the period of contraction the closing of the end makes comparatively slow progress, although a gradual narrowing of the opening does take place beginning immediately after the cut is made. But when the tentacle begins to extend, the cut end closes in very quickly and with continued extension the nipple is formed. Thus the closing appears, for the most part, to await the extension of the tentacle and then takes place within a few seconds. In all cases, then, there is to be seen a rapidity of closing which is inconsistent with the view that the process depends upon amœboid motion or that it is of the nature of growth changes. In view of the known histological structure of these tentacles it can hardly be questioned that this rapid closing of the cut end is due to contraction of circular muscle fibers.

In the second place, the appearance of the tissues in the nipple and around the base of it is such as may be seen in any contracted part of an uninjured normal tentacle. The tentacle wall in extension is yellowish and translucent. When contracted it is whitish and opaque, and the more it is contracted the greater is the degree of whitishness and opacity. The white opaque nipple indicates extreme contraction of tissues. Passing proximad over the surface of the end of the tentacle the whitishness and opacity gradually diminish, merging into yellowishness and translucency just where

the hemispherical end wall of the stump merges into the lateral wall. These conditions support the view that the tissues of the hemispherical end are in a state of muscular contraction, the contraction being greatest at the base of the nipple and approaching nil at the equator of the hemisphere.

Thirdly and finally, the fact that the nipple, having once been formed, may disappear and reappear in the course of the alternate contractions and extensions of the tentacle clearly indicates the muscular nature of the closing process. In this connection the mechanism of contraction and expansion of tentacles must be considered. In a fully extended normal tentacle there is apparently complete relaxation of both the longitudinal and the circular muscle fibers. Increase in length is not necessarily at the expense of diameter as it is in Hydra or in the body of the earthworm. The explanation of this difference lies in the fact that in the earthworm the fluid content of the body is practically constant, whereas in the actinian the contents of the tentacle may vary owing to its being merely an appendage whose lumen is in free communication with the much more voluminous gastro-vascular cavity.

The fully extended tentacle is at its maximum diameter as well as at its maximum length. In this relaxed condition its distension is secured by the gentle internal fluid pressure. Shortening of the tentacle results from contraction of the longitudinal fibers, the circular fibers apparently remaining relaxed, for, at least during moderate contractions, there is no perceptible change of diameter. But in extreme contraction there may be contraction of circular fibers also. In the extension of a tentacle the longitudinal fibers are relaxed and in the normal tentacle there is usually no obvious contraction of the circular fibers. But in a detached piece of tentacle or in a piece of tentacle ligated onto the hydrostatic tube there may often-not always-be seen a marked diminution of diameter as the tentacle elongates (see Figs. 9 and 14a'). In the ligated piece this circular contraction sometimes yielded to a little internal pressure, but sometimes it did not. The behavior of a normal tentacle indicates that the conditions of extension are, first, general relaxation of all muscle fibers, and, secondly, internal fluid pressure. Child ('04b) considers at length the importance of

internal fluid pressure in relation to the form and activities of Cerianthus.

As previously mentioned, the pieces of tentacle which had been detached from the column never exhibited a state of complete relaxation. Their changes of form were always relative to a certain and considerable degree of persistent contraction in all dimensions. This contraction was only partially, never completely, overcome by the artificial application of internal pressure, thus proving that the failure to attain full expansion was not due merely to the absence of the normal pressure of the gastro-vascular fluid, but rather to some lasting change in the tissues themselves occasioned by the cutting of the tentacle. However, as regards the behavior of the tissues at a distal cut end, whether the stump of tentacle was attached normally to the column or ligated to the pressure tube or not attached to anything, there was in one respect entire uniformity, namely in this,—that under conditions of general extension the distal opening became narrowed or quite closed, while under conditions of general contraction the cut end became more broadly open. These rapid changes in the area of the opening certainly depend upon muscle action. Furthermore, this behavior indicates that a band of circular fibers at the cut end is working independently of other fibers and, to a certain extent in opposition to them. Thus, when a tentacle elongates the circular fibers in general may not contract, as appears usually to be the case in a normal tentacle, or they may contract as sometimes noted in detached fragments of tentacles. However that may be, there is at a distal cut end a band of circular fibers which invariably contract when the tentacle elongates and which may relax more or less when the tentacle shortens. The more the extending tentacle becomes dilated under general relaxation of circular fibers, the more tightly closed is the distal end. The nipple, then, results from the extreme contraction of a distal band of circular muscle fibers.

The experiments have shown that in the course of a few days the nipple disappears, the cut end becomes completely closed, even lacking the terminal pore characteristic of a normal tentacle, and the whitish opacity indicative of contracted tissues fades away.

These changes involve something different from muscle action. There is not the least evidence of production of new tissue. It is old tissue which finally closes the cut end—the same tissue which by virtue of its own muscular contraction had temporarily closed the end during the extension phases of the tentacle. Some readjustment of the tissue elements takes place with the result that a layer which originally, in absence of muscle contraction and in presence of internal pressure, possessed a cylindrical surface becomes changed so that under similar conditions of tension and pressure it possesses a spherical surface. The process of this readjustment is a gradual and slow one. first effects may be seen in the contracted stump or fragment of tentacle within a few minutes after the cutting. The cut edges begin to draw inward toward the axis of the tentacle so as to diminish the area of the opening, but so slowly that the change is imperceptible except as conditions at intervals of an hour or two are compared. This slow and gradual reduction of the distal opening appears to be entirely independent of the muscular activities which have been described above. The tentacle passes through its alternate phases of contraction and extension (extended most of the time, if not disturbed), the cut end during extension being virtually closed by means of the nipple, while during contraction it may be more or less widely open, but less widely open the longer the time which has elapsed since the act of cutting. Eventually, as a result of this rearrangement of the tissue elements, the cut end becomes structurally closed and, so far as muscular tension is concerned, conditions in the tissue which closes the end are uniform with those elsewhere in the tentacle—that is, the closure of the end is no longer dependent upon special muscular contraction in that region. In the attached tentacle the progress of these changes is obscured owing to the fact that the tentacle, if not stimulated, remains constantly extended with its cut end always closed by the nipple. Yet these readjustments just as surely take place there, as is shown by the gradual and complete disappearance of the nipple and of the zone of muscle contraction around During the progress of these changes the closure of the end depends less and less upon muscle contraction, and more upon fixed structural conditions

Almost immediately after cutting a tentacle there is to be seen a slight but distinct inbending of all the cut edges. In its abruptness this change differs from the later slow closing of the cut edges, which may require as much as a day for completion. It certainly appears to be quite distinct from the muscular contractions which ensue. This abrupt initial inbending is similar in nature to what has been seen in so many two-layered organisms, both embryo and adult, where cut walls promptly bend so that ectoderm is on the convex side of the curve, a condition which suggests some preëxisting inequality of tension in the two body layers.

When a transverse slit is cut in the side wall of a tentacle the closing of the wound takes place in much the same way as when a piece is removed. There is immediately a muscular contraction of the tissue around the slit as a result of which the slit is virtually closed. The appearance of the tissue around the slit is as if a ring of muscle fibers surrounding the slit was in a state of contraction. However, this appearance may very well result from the local contraction of both the longitudinal and circular muscle fibers in the near vicinity of the cut. The sharp bending of the tentacle, which looks so beautifully protective, is perhaps nothing more than an incidental result of the local and one-sided contraction of tissues. Then, during the next day or two, there takes place a slow structural closing of the opening. The muscular contraction diminishes as this closing progresses. There is no external evidence of formation of new tissue.

Comparing the behavior of the transversely cut tentacle of one of these large actinians with the behavior of the similarly cut trunk of Hydra it will be seen that the processes of closing a cut end are to a certain extent very similar in the two cases, if not identical. In both there is an immediate, although slight, inbending of the wall at the cut edge, probably due to difference of tension in the body layers. Then ensues a slow continuous inbending of the wall at the cut end, the opening being thus gradually diminished until the end is completely and structurally closed. (In the attached tentacle this process is obscured by the presence of the nipple.) In Hydra the closure may be completed within half an hour. In a Condylactis tentacle it requires several hours or even a day.

This difference in time is consistent with the difference in the cross sections of the two structures. The diameter of the tentacle in Condylactis is, in rough terms, from fifteen to twenty-five times as great as that of the trunk of Hydra viridis. In both cases the cut end becomes closed by old tissue; there is no proliferation of new tissue. In the introduction to this paper I have maintained that the closing in Hydra is dependent upon cell activities akin to amœboid motion. The structural closing of the distal cut end of an actinian tentacle is certainly due to some rearrangement of the tissue elements and any such rearrangement, necessarily involving changes in the form and relative position of cells, must depend upon cell activities closely allied to amœboid motion. In so far, therefore, the processes of closure of the very simple stem of Tubularia, the more complex trunk of Hydra, and the still more complex and enormously larger tentacle of Condylactis are probably reducible to the same terms—changes in the form and position of preëxisting tissue elements. But in the total reaction of the large actinian tentacle there is to be seen one feature which is not exhibited by the tubularian stem or by Hydra, namely, the immediate but temporary muscular closing of the cut end. In this feature of the reaction is afforded a very effective muscular control of the cut distal end pending the completion of the slow process of structural repair.

It seems reasonable to suppose that there is some advantage to the organism in the prompt closing of the injured end of a tentacle. The loss of a single one of the many tentacles possessed by these actinians cannot be a serious matter. But if a single cut tentacle were allowed to remain freely open it would not be long before the escape of gastro-vascular fluid and the release of the normal internal pressure must lead to a more or less serious disturbance of the entire organism. The prompt muscular closing of the cut has the result, very important to the organism, of obviating this general disturbance or at least reducing it to a minimum, while—doubtless a much smaller advantage—the injured tentacle itself is almost immediately restored to a condition which, so far as its general reactions are concerned, appears physiologically normal, although no structural repair of the injury has yet been made.

The insertion of an injured tentacle into the gullet seems to me of doubtful significance. It might be regarded as a means of closing the cut end of the tentacle, but as such the act would appear to be a superfluous one, since the cut end is already effectively closed by the nipple. In structures so small of cross section as the tubularian stem and the trunk of Hydra the consequences of exposing the gastro-vascular cavity cannot be so serious as in the incomparably larger actinian. There is no evidence that internal pressure plays the important rôle in Hydra that it does in the large actinian. Further, in these smaller structures the permanent repair by the rearrangement process is completed within a comparatively short time. Accordingly there would seem to be no need of such a provisional control of the wound as we have seen to be operative in the large actinians. In Tubularia, at least, a muscular apparatus capable of such control is lacking.

The experiments which I have described have shown that the behavior at a distal cut end of a detached tentacle is the same. so far as the essential features are concerned, as when the tentacle is normally attached to the column. The detached tentacle in response to a distal cut exhibits the immediate reaction of muscular control of the distal cut end as well as the slower structural readjustment by means of which the permanent and non-muscular closure is effected. There are, as we have seen, two respects in which the tentacle is affected by detachment from the column. In the first place there is some physiological difference between the tissues of a normal tentacle and those of a detached tentacle, as shown by the persistent contraction of the detached tentacle. Whether this physiological change is caused by the stimulus of cutting or by the presence of a cut surface or whether it is possibly due to the absence of some inhibitory effect normally exerted upon the tentacle by the column, I am not able to say. In the second place there is a purely mechanical difference between detached and normal tentacles in that the former lack the normal internal fluid pressure. These two differences are accountable for certain differences in the details of the behavior of attached and detached tentacles. Otherwise reparation takes place in detached tentacles precisely as it does in attached tentacles.

These reparative responses, then, are potentially of local character. They are not necessarily dependent upon the organism as a whole individual, but they may be initiated and carried out by the tentacle tissues in the near vicinity of the plane of cutting. We have here a very clear instance of a noteworthy peculiarity of organisms in general, namely, a purely local response to change of conditions—a response effected entirely independently of any immediate action of the organism as a whole—and yet one which is admirably adapted to the needs of the whole organism. It can hardly be imagined that the repair of the distal cut end is of any advantage to the detached tentacle, for the proximal cut end (in Condylactis) is not readily repaired and the fragment of tentacle under ordinary conditions is capable of maintaining life for only a few days at the most. In the light of regeneration experiments on the lower invertebrates it would seem fairly certain that even under more favorable conditions there could be no regeneration from the fragment of tentacle. Yet it is certainly true that the closing of the cut end of the fragment tends toward the independent existence of the fragment. If it were to continue to live indefinitely the closing or healing of the cut ends would be the thing of primary importance for it. The behavior of fragments of tentacles might be described as an attempt, albeit an ineffectual one, to survive. Certainly not, in a narrow sense, an "attempt" having origin in the particular individual of my experiment, but, in a broader sense, an expression of those fundamental and distinctive properties of living substance which tend toward persistence and increase of organisms.

The survival of actively motile fragments of Aiptasia tentacles for a week or more (see p. 228) suggests the possibility that under more favorable conditions of experimentation than I was able to arrange with the time and means at my disposal the life of such fragments might be prolonged indefinitely. Indeed, is it not conceivably possible that by use of a suitable nutrient medium and by chemical stimulation growth activities might be aroused in such fragments and regeneration of some sort induced? So far, too, as the utility of reparation to the fragment itself is concerned, it is possible to regard the matter in either a retrospective

or a prospective light. The present ineffectual attempt at survival may a semnant of a phylogenetically ancient capacity for complete regeneration. Or, it conceivably affords a basis upon which the power of regeneration may subsequently be grafted. The latter possibility is a much less likely one in view of the general tendency of evolution toward more complex structural conditions and the inverse correlation of structural complexity and capacity for regeneration.

The foregoing views regarding the significance of the behavior of fragments of a tentacle may well be abandoned, however, in favor of a much simpler interpretation. Whether or not the reparation of the fragment is in any sense, however remote, of utility to the fragment itself or to the species, I think that the behavior of the fragment becomes to a certain degree intelligible when we view the detached tentacle merely as a fragment of the actinian and with no reference to its own fate. We have seen that the reparation process involves two phases; first, the provisional muscular control of the cut end and secondly the definitive structural closing of the end. Considering the first of these two phases and keeping in mind the character of the actinian organization, it seems to me not surprising-indeed, to be expectedthat the fragment of tentacle should behave like the attached tentacle. The actinian shows a rather low degree of specialization of parts of the body as compared with higher Metazoa. is a two-layered sac having certain appendages, which are merely hollow diverticula of the sac. All sections of the body wall, wherever taken, show the same arrangement of layers and cavities. There are no localized central nervous organs. The fundamental cœlenterate features of the actinian organization are repeated throughout column and tentacle. A piece of tentacle, then, is indeed a fragment of the actinian—that is, of the organism—but it is not a fragment of the organization, by reason of the fact that it embodies the essential features of the entire organization. The distal cut end of a detached tentacle, therefore, differs from the distal cut end of an attached tentacle merely as to the quantity of a certain kind of organization lying proximad of them. It seems to me likely that the action of the muscles at the distal

cut end of an attached tentacle are under nervous control, and it may be that the nerve mechanism of the entire actinian participates in that control. Even if that be true, we may well suppose that the fragment of tentacle exhibits the same kind of muscular activity by virtue of possessing the same kind of nerve mechanism, although in less quantity.

This reparative behavior of excised tentacles calls to mind the observation of Parker ('96) on excised tentacles of Metridium. He stimulated excised tentacles with meat juice and found that they bend in a direction corresponding to the original position of the mouth in relation to the tentacle. Thus, it appears that "each tentacle has within itself a complete and independent nervous and muscular mechanism capable of carrying out normal responses" (p. 112). However, in the responses both to cutting and to stimulation by food we see, coexistent with a high degree of autonomy in the tentacle, a certain necessary relation of it to the whole organism. This relation continues to be expressed even after the tentacle is separated from the rest of the animal. In such behavior of a fragment the idea of the whole is expressed, although in much less detail, yet quite as vividly as if the fragment had regenerated the whole actinian. Whatever may be the nature of the mechanisms underlying these, in a sense autonomous, responses which are carried out by fragments of animals, can we avoid the conception that somewhere in the total life-history-in ontogeny or phylogeny—the individuality or totality of the organism or some "original principle of unity" (Lillie, '06) has been a cause operative, in a way which is beyond our knowledge, toward the establishment of such mechanisms and the relations which their activities express?

Turning now to the second phase of the reparation process, namely, the slow structural readjustment by which the cut end is permanently closed, I believe that we have here to deal with a very fundamental and primitive property of the tissues concerned. In the Metazoa in general the early ontogeny includes a two-layered condition. Of these two layers the ectoderm gives rise to the definitive outer layer of the body, while the chief derivative of the entoderm is the enteric tube. Experiments which have

been made upon a large number of animals and representatives of various groups—chiefly cœlenterates, worms and vertebrates agree as to the following results. If a portion, not unreasonably large, of the surface of the animal is denuded of its normal ectodermal layer, the lost outer layer is quickly replaced through the agency of the neighboring ectoderm. The process certainly does not depend in any way upon contraction of muscle cells, it is probably not under nerve control, and it does not necessarily involve cell division. It consists rather of a rearrangement of existing ectoderm cells, a concentric centripetal movement of the edges of the layer-in the earthworm, at least, an independent migratory activity of epidermal cells. Similarly, if the entodermal cavity is exposed its walls tend to close upon themselves and assume a deep position with reference to the ectoderm. In more complex organisms, such as annelids, this may be wholly or partly due to the action of an investing muscle layer. The significant fact is that the same thing happens in such a simple animal as Hydra where the muscle processes can hardly be accountable for it, and-still more significant—in two-layered embryos. Thus, the twolayered condition having once been established, it is then the primary function of an ectoderm cell to be on the outside. More than that, it is the function of ectoderm cells collectively to cover all outside surface. The denuding of a bit of surface of its proper layer of ectoderm appears to demonstrate a special affinity of ectoderm cells for the exposed surface of deep tissues.

The normal perforations of the body wall arising by the commonly observed processes of invagination, evagination and rupture are determined we do not as yet know how. But artificial perforations of the body wall are usually promptly closed primarily through this capacity of the body-layer elements for positional readjustment. This capacity is inherent in the tissues. It does not matter, therefore, whether the fragment of organism be a major one (the actinian minus a bit of tentacle) or a minor one (the bit of tentacle which has been cut off), for in both cases the body layers set about the structural repair of the breach in the same way.

In all of these reparative readjustments the elemental process appears to be of the nature of amœboid motion.

Finally, then, in the reparation of these actinian tentacles I believe that we may distinguish two totally different phenomena. The structural closing of the wound is due, not to any special process peculiar to actinians but, upon the contrary, to certain fundamental properties of the elements of the two primary body layers of Metazoa-properties to which these organisms must owe their very origin and existence. In the other phenomenon, the temporary muscular control of the wound, we see a much narrower and more highly specialized kind of activity. In just this particular method of operation it is perhaps peculiar to the actinians. It is very clearly an adaptation to the circumstances that wounds are necessarily of large area as compared to the thickness of the body layers and that to await the closing of such wounds by the slow primitive process of cell-rearrangement would involve delay more or less disadvantageous if not positively dangerous to the organism.

The fact that the fragment of tentacle repairs itself in the same way that the attached tentacle does is not necessarily significant of any peculiar autonomy of the structural units of an organism. It results from two conditions; first, that the fragment includes both body layers with their distinctive and ever inherent primitive properties; and secondly, that the peculiar actinian features of organization in general—more particularly in this connection the nerve-muscle arrangement—do not involve sharp localization of tissues into spatially restricted organs, but rather a diffuse distribution of the more specialized structures and their corresponding functions.

Finally, if we compare the actinian tentacle with similar coelenterate structures—the tubularian stem and Hydra—upon the one hand, and with the earthworm upon the other, it may be observed that the actinian tentacle possesses a somewhat greater complexity of structure than the tubularian stem or Hydra, correlated with its greater size. This greater complexity is seen chiefly in the differentiation of definite systems of muscle fibers. Corresponding to this particular additional complexity of structure we find in the actinian tentacle a feature of behavior, namely, the provisional muscular control of the wound, which is superadded to the be-

havior exhibited by the smaller and simpler coelenterate struc-In the earthworm, with much greater diversity of structure, the reparation process is still more complex than it is in the actinian tentacle. The immediate closing of the wound in the earthworm is effected by contraction of the body-wall musculature and the formation of the cicatricial plug. Therefore these acts are, in a sense, the physiological equivalent of the muscular control of the cut end of an actinian tentacle. The covering of the surface of the earthworm cicatrix by migration of epidermal cells is represented in the actinian tentacle by those internal readjustments which bring about the relief of the muscular control of the cut end and effect the structural closing of it, for these are the processes which, similarly in the two organisms, reëstablish normal relations and conditions of tissues. Only the impaired form then remains to be corrected, so far as the organisms are able, by regeneration.

It must not be inferred that the foregoing discussion purports in any sense to explain what happens in the actinian tentacle. My aim has been to describe and to analyze the events in the hope that the so doing may enable us to see a little more clearly the nature and scope of the phenomena which remain to be explained.

#### POLARITY

Thus far we have described the behavior of only the distal cut end of a tentacle. If now we consider what happens at a proximal cut end we shall immediately find some differences which are noteworthy. The immediate reactions of an excised tentacle of Condylactis are described on p. 203. The characteristic features of the behavior of the proximal cut end are as follows. The cut edges bend inward slightly within a few seconds after the cutting. This inbending very gradually becomes more decided and as it does so the walls of the proximal end develop deep folds or wrinkles. Figs. 6a and 6b show the condition of proximal cut ends about one and a third hours after the cutting. Fig. 12 represents the condition of three consecutive fragments of a single tentacle about one hour after the cutting. Fig. 13 shows two pieces of a tentacle

as they appeared two hours after the cutting and in Fig. 14 are represented three consecutive pieces of a single tentacle six hours after cutting. In one experiment a fairly large tentacle of Condylactis was cut off near its base and then its distal end, representing about one-fifth the length of the tentacle, was cut away. The conditions of both the larger and smaller parts of the tentacle at eighteen hours after the operation are shown in Fig. 15. In another case about three-fourths of a tentacle was excised and this piece was then cut in two midway of its length. Twenty-four hours later the proximal fragment was fixed in mercuric chlorid. the fixed condition the form of the fragment was practically the same as when alive and strongly contracted. An end view of the proximal end of the fixed piece is shown in Fig. 7. These figures serve to illustrate two features which were fairly conspicuous and constant. First, the wrinkling is entirely confined to the proximal ends, while the distal ends are quite smooth. Secondly, the amount of wrinkling is decidedly greater at proximal cut surfaces situated nearer the base of the original tentacle than at those nearer the tip. This latter fact I believe to be connected with the fact that the diameter of the tentacle is much greater near the base than it is near the tip. The further history of fragments of Condylactis tentacles is this. The proximal end did not in any case become closed. After the first day the walls about the proximal end exhibited little change. They remained sharply infolded and much puckered until the fragment underwent dissolution, which occurred from two to four days after the cutting. Usually during the second day masses of broken down entoderm were intermittently discharged from the open proximal end, never from the distal end.

The facts cited serve to show that there exists a marked difference in the behavior of proximal and distal cut ends. To demonstrate this difference as clearly as possible I made a series of experiments in each of which a single excised tentacle of Condylactis was transected into several pieces, generally three, and a careful study was made of the corresponding proximal and distal cut ends. (By "corresponding proximal and distal cut ends" I mean to indicate the two cut ends produced by a single transection; that is, the distal end of the piece lying proximal of the plane of cutting

and the proximal end of the piece lying distad of that plane.) The results of this study may be summarized as follows.

During the first half hour after the cutting the behavior of proximal and distal cut ends was not strikingly different. The proximal ends showed a somewhat greater tendency to wrinkle than the corresponding distal ends. As already noted, the wrinkling was more marked in the pieces nearer the base of the tentacle. Further, the tendency of the pieces to collapse was much greater in pieces from the base of the tentacle than in those from its tip, a difference which is evidently due to the difference in diameter at base and tip. The openings at all the cut ends were diminished in area by the characteristic inhending of the cut edges. In many cases it could be seen that a distal opening was slightly smaller than its corresponding proximal opening. Immediately after cutting, the cut edges are slightly ragged and more or less angular in outline as a result of the pressure of cutting. In some cases even during the first half hour a difference arose between proximal and distal ends in that a distal end became more nearly smoothly circular in outline than its corresponding proximal end. We have already noted that excised fragments of tentacles ordinarily do not exhibit the nipple condition except under the influence of a little artificially applied internal pressure. In these polarity experiments no attempt was made to apply internal pressure becauseI desired to introduce no unnecessary disturbances into the experiments in order that conditions at proximal and distal cut ends should be as nearly as possible identical so far as outside factors were concerned. Nevertheless there were many cases in which a fairly distinct nipple did appear at the distal end of the middle one of the three pieces into which a tentacle was cut, but there was no case where a nipple formed at the distal end of one of the most proximal of the three pieces. When the tentacle was cut into two pieces a nipple often formed at the distal end of the proximal fragment. In a few cases, too, the proximal end of the most distal piece assumed a nipple-like form resembling, therefore, the distal ends of those middle pieces which formed nipples. All of these proximal nipples were of comparatively brief duration. Gradually becoming less marked, at the end of an hour they had disappeared, 226 Herben

allowing the proximal end to become wide once on 1 show 1:1 t in any case reappear.

During the second half hour after the operation the corresponding proximal and distal cut ends became distinctly different in two respects. First, a proximal cut end was much wrinkled, while its corresponding distal end was perfectly smooth. The most distal fragment of the tentacle sometimes afforded an exception to this statement in that its proximal end, being of small diameter, wrinkled only slightly or not at all. In some cases the distal end of the basal fragment of the tentacle became more or less wrinkled, but here the wrinkles were only temporary, and they were usually quite smoothed out by the end of the first hour. Secondly, the hole at any distal end was much smaller and more smoothly circular in outline than the hole in the corresponding proximal cut end. Fig. 12 shows a typical case one hour after the cutting. The three fragments represented in the figure are consecutive pieces of one tentacle and they are drawn in their original axial relations to one another. The pieces are shown in a somewhat contracted condition because they rarely became much extended. The appearance of a similar set of three fragments six hours after the cutting is represented in Fig. 14, and this case is typical of all the experiments. The basal piece is shown at a in the usual contracted condition, while at a' it is represented in partial extension. Extension in this case seemed to involve contraction of circular fibers. At this period may be noted three conspicuous differences between corresponding proximal and distal cut ends. First, proximal ends are still more or less wrinkled and distal ends are not. In some cases wrinkles did occur within the distal half of a fragment but these wrinkles were merely extensions of the proximal wrinkles into the distal half of the piece. Such wrinkles never extended quite to the distal surface and never encroached upon a smooth zone immediately surrounding the distal opening. Secondly, every distal hole is conspicuously smaller than its corresponding proximal hole—that is, much smaller than the proximal hole of the next fragment distad. The third difference, and one which has not previously been noted, is this.—A distal cut end, except when the fragment is strongly

contracted, whereas smoothly to a sharp tip whose diameter is very nearly the same as that of the distal pore, whereas the corresponing proximal cut end is broad and blunt, its walls are abruptly inturned, and its comparatively large opening is irregularly outlined. The general form of the fragment, therefore, is that of an irregular cone, and the irregularities are much greater toward the base of the cone. In all of these respects the most distal fragment shows rather less marked polarity than the others.

The conditions just described persist for many hours or for a day or two without noticeable alteration except for the continued diminution of the distal pore which, one day after the cutting, is usually very minute or not evident at all.

Three tentacles were excised and allowed to lie undisturbed for two days. They were then transected about midway of their length. The behavior of proximal and distal ends of the fragments was substantially the same as when transection followed immediately upon the excision of the tentacle.

Experiments similar to those just described were made also upon tentacles of Aiptasia. The results, so far as polarity is concerned, were much less striking than in Condylactis. The comparatively small diameter of the tentacles of Aiptasia and their persistent and excessive contraction after cutting made them much less favorable objects for the expression of any polar tendencies which they may possess. In most cases the corresponding proximal and distal cut ends exhibited no differences which were marked enough to be considered significant. But in a few instances there did appear unmistakable differences which were similar in nature to those which have been noted in Condylactis. Fig. 16 represents a tentacle of Aiptasia cut into two pieces. The distal end of the proximal fragment (a) is slightly tapering at its tip, while the proximal end of the distal piece (b) is broad and blunt. It should be remembered that the normal tentacles show only a slight decrease in diameter from base to tip. Therefore the smaller tapering distal tip of the proximal fragment and the larger blunt proximal end of the distal fragment represent regions of the original tentacle which originally had practically the same diameter. In some cases, too, it was noted that at a distal cut end there was a

deeper recession of the entoderm and a thicker covering of ectoderm than at the corresponding proximal cut end. These Aiptasia tentacles, when cut, contracted to such an extent that their cavities were nearly or quite obliterated. Therefore neither proximal nor distal cut ends were actually open in the sense that they were in Condylactis.

In connection with this study of fragments of tentacles of Aiptasia, I was interested in repeating the observation made by Parker ('96) upon Metridium. He found that an excised tentacle "has a strong resemblance to an independent organism, and by means of its cilia glides slowly through the water with its base forward, a fact in accordance with the observation that on attached tentacles the current produced by the cilia moves from base to tip" (p. 110). Fragments of Aiptasia tentacles remained alive for seven or eight days moving about slowly and continuously with proximal end foremost, and showing occasional spontaneous contractions. Many of the fragments were slightly curved and therefore moved in circles. The cut ends appeared closed but it is probable that the proximal end, at least, was not perfectly closed for, beginning usually a day or two after the cutting, there were occasional discharges, through the proximal end, of substance from the interior of the tentacle. This substance consisted chiefly of the brown Zoöxanthellæ, to which the tentacle owes its color. Sometimes these bodies were discharged in a continuous stream for several minutes, as if by the action of the entodermal cilia. As a result of these discharges the fragment faded in color and diminished in size. By the third or fourth day the fragment had become quite colorless and was reduced to a half or even to a fourth its original size. Some of the later discharges were of colorless material. This was probably disintegrated entoderm, yet the ectoderm was perfectly intact and its cilia continued to beat vigorously until within a few hours of the final disintegration of the fragment. The fragments of tentacles of Condylactis did not move about, doubtless owing to their relatively greater bulk. They disintegrated sooner than fragments of Aiptasia, probably because of the wide open proximal end.

In looking for these expressions of polarity, it occurred to me

to secure a piece of tentacle onto the hydrostatic tube in orientation the reverse of that of the preceding experiments—that is, to tie the distal end of the fragment onto the tube, leaving the proximal end free. I attempted to do so first with tentacles of Condylactis. After many trials I abandoned the attempt owing to the fact that the manipulation of the distal end of the excised tentacle induced such excessive contraction that the tentacle was reduced to a shapeless lump into which the glass tube could not possibly be inserted properly. But the very failure of this attempt demonstrated a striking physiological polarity, since it appeared that irritation of the distal end of a tentacle caused much more severe contraction than similar irritation of a proximal end. I shall refer to this matter again. I then tried the same experiment on tentacles of Aiptasia. Of many attempts, only one succeeded. At the expense of much mutilation of the distal region of a large tentacle I finally secured it to the end of the glass tube. Within five minutes the tentacle began to stretch out a little although so sharply contracted in diameter that its lumen must have been practically closed. I then began applying internal pressure. Moderate pressures produced no change in the appearance of the tentacle. But under a pressure of about 80 mm. the contraction was partially overcome and the tentacle became distended. It elongated considerably and also swelled out laterally, but its free proximal cut end remained so much contracted that only a small pore allowed the contained water to escape. The form of the tentacle under these conditions is shown in Fig. 17. A few minutes later I cut a small fragment off the proximal end, thus obtaining a new cut surface lying at a slightly different region of the tentacle. Upon application of internal pressure the conditions of Fig. 17 were repeated. The removal of a second proximal fragment transferred the cut surface into a still more distal region of the tentacle and with perfect repetition of the preceding behavior.

In this experiment the distention of the tentacle was certainly nothing more than the direct mechanical effect of the water pressure. But why did the free proximal end remain contracted? Is this condition in any way comparable to that of the free distal cut end which closed and formed a nipple? I believe that it cer-

tainly is not, for the following reasons. The form of the reversed tentacle, as shown in Fig. 17, is exactly what would be expected as the direct result of the mechanical pressure. It does not require any special contraction of the tissue in the vicinity of the free proximal end. The tentacle is a hollow cylinder whose wall consists of very thin and elastic tissue. Previous to the introduction of the pressure the whole tentacle was in a state of uniform and extreme contraction. Slight pressures, such as induced extension of a piece of tentacle attached to the hydrostatic tube by its proximal end, produced no visible effect in this case. By pressure of 80 mm. the contraction of the tissues was forcibly overcome. The tentacle became more or less distended throughout its entire length. But directly at the free proximal end there was less distention simply because there was less pressure owing to the proximity of the opening through which the contained water escaped. On the other hand, the form of the piece of tentacle attached to the pressure tube by its proximal end is not such as would result passively from internal pressure. In this case extension often occurred under pressure too slight to produce any appreciable stretching of the tissues, and, further, the complete closing of the free distal end and the formation of the nipple cannot possibly be direct mechanical results of the internal pressure. We may safely conclude, then, that under the conditions of the experiment with the hydrostatic tube the free distal cut end executes certain special contractile activities which result in the forming of the nipple and the closing of the end while the rest of the tentacle is in a state of more or less relaxation. Its behavior is like that of the free distal cut end of a tentacle which is normally attached to the column. But the free proximal cut end, under the same conditions of experiment, does not exhibit any intrinsic activity which differentiates it from the rest of the tentacle. The entire tentacle, in a state of persistent and extreme contraction, exhibits, like any inanimate structure, only passive changes of form as the direct mechanical effects of internal pressure.

In the behavior of a normal tentacle certain expressions of polarity are evident. One is to be seen in the directive stroke of the cilia. Another, and a very conspicuous one, is afforded by the reactions of the tentacle to tactile stimuli. Under a tactile stimulus of appropriate intensity, the region of the tentacle situated proximad of the point of application of the stimulus may be caused to contract very considerably while the distal portion of the tentacle contracts comparatively little or not at all. Nagel ('94) and Torrey ('04) do not mention this form of reaction as occurring in the actinians, which served as the subjects for their experiments with tactile stimulation. The conditions in more detail are as follows. The experiments were made upon tentacles of Condylactis.

If a sharp needle point be applied very gently to the side wall of a tentacle the result is a purely local longitudinal contraction. The contraction involves a zone or ring of tissue less than a millimeter in width, although its limits are not sharply defined, and extending transversely completely around the tentacle. The shortening of the tentacle caused by this contraction was doubtfully perceptible, but the zone of contraction was plainly evident because of its whitish opacity. It remained visible for two or three seconds and gradually faded out. Owing to this persistence of the contraction, it was possible by touching several points upon the tentacle in rapid succession to bring about the simultaneous presence of as many zones of contraction.

With slightly more vigorous stimulation the local contraction zone appeared as before, but in addition to that there was a more or less definite contraction of all that part of the tentacle lying proximad of the point of contact, while very often I could not detect the least contraction in the distal portion of the tentacle.

Under still more vigorous stimulation the entire tentacle contracted, but even in this case it often appeared to me that the proximal part of the tentacle contracted more promptly and in greater degree than the distal portion. The local zone of contraction could sometimes be seen when the entire tentacle contracted. With the more vigorous stimulation the local contraction persisted for a longer time. Often the whitish zone at the place of stimulation was clearly evident even after the tentacle had otherwise recovered from a condition of general contraction. In cases of extreme general contraction the local contraction was usually indistinguishable. Although I did not employ any means of measuring pre-

cisely the intensity of the stimuli and the degree of the contractions, it was quite evident that the amount of contraction varied with the intensity of the stimulus—more particularly, that the stimuli which caused contraction on only the proximal side of the point of contact were upon the average less intense than those which caused contraction of the whole tentacle.

It is apparent, then, that there is a certain polarity in the effects of a tactile stimulus, in that motor responses are produced upon the proximal side of the point of contact more readily than upon the distal side of it. Some features in the behavior of cut tentacles are in accord with these facts. When a piece of tentacle is excised the stump of the tentacle contracts proportionately much more than the piece which is cut off. If an excised tentacle is cut into two pieces the proximal piece contracts more and shows much more conspicuous motor disturbances than the distal one. (See p. 203.) Still further, I found it comparatively easy to ligate the proximal end of a tentacle onto the hydrostatic tube, but my efforts to ligate a distal end onto the tube were, with one exception, failures on account of the excessive contraction induced by cutting and manipulating the distal end.

This behavior of the tentacle is strikingly similar to the behavior of worms as shown by the experiments of Norman ('00), who found that transection of a worm was followed by conspicuous motor disturbances in the portion of the animal posterior to the cut, while the anterior portion exhibited only slight response to the cutting, or none at all.

In the beat of the cilia and in the reactions of tentacles to tactile stimulation and to cutting, we see a physiological polarity Underneath it may lie some as yet undiscovered structural polarity. In the different forms of proximal and distal cut ends of fragments of an excised tentacle we have an instance of morphological polarity. Is there any significance in the fact that proximal and distal cut ends of a deteched portion assume different forms? It seems to me obvious that there is. The distal cut end tends to assume the form and condition of the tip of a normal tentacle. This tendency is to be seen in the tapering form of the distal cut end and in its prompt closure, which is effected by the same

means that an attached tentacle employs for the repair of its distal cut end. It may be admitted as possible that in the conditions at the distal cut end of a fragment we see merely an accidental and meaningless resemblance to the tip of a normal tentacle. There may be absolutely no connection between the two sets of facts. From this point of view the behavior of the cut tentacle is quite unintelligible. But on the supposition that the form of the distal cut end of the fragment and the conditions at the tip of the normal tentacle rest upon some common basis in the organization, the behavior of the fragment comes to possess a certain meaning for The multitude of facts which have been established demonstrating the plasticity of organisms and their widespread capacity for regulation toward some specific normal form justify us, I believe, in accepting the latter view—the one which does give the facts a significance. The behavior of the proximal end of the fragment is not inconsistent with this view. The normal condition of the proximal end of the fragment is that of attachment to a similar structure. Obviously the fragment cannot in any way regain this normal condition except by regenerating a new actinian at the proximal cut surface, and it is quite incapable of doing that. The tissues of the tentacle wall, by virtue of their primitive property of inbending, make some attempt, so to speak, to close the proximal cut end, but the attempt to close an opening which is so large in relation to the thickness of its walls is an unsuccessful one in the absence of the aid received from muscular contraction which plays so important a part in the closing of a distal end. Except, then, for this abortive attempt at closing, the proximal cut end does nothing. Even if such muscular contractions as facilitate the structural closing of a distal end were to take place also at a proximal end, is is a question, I believe, whether the proximal end would then become structurally closed. Its normal condition is to be open. Further experiment upon tentacles of small diameter might answer this question.

It has been mentioned that in a few cases the proximal end of a very short piece from the extreme tip of a tentacle assumed temporarily a nipple-like form. This brings to mind cases of regeneration with reversed polarity (heteromorphosis) as seen in the

earthworm and planarians, where a head-like growth may take place at the posterior surface of a very short anterior piece of the worm or a tail-like growth may appear at the anterior surface of a short posterior piece. Just as the capacity for regenerating a head in normal orientation is most strongly present in the extreme anterior region of the worm, so in the actinian the nipple is formed most promptly and constantly at distal cut surfaces near the tip of the tentacle. In both cases there is doubtless a connection between the fact that a certain capacity is concentrated in a particular region of the organism, and the fact that this capacity occasionally expresses itself there in an orientation which is the reverse of the usual one.

The morphological polarity seen in the tentacle becomes most striking when we consider the fact that every region of the tentacle (possibly excepting the extreme tip) possesses the capacity of behaving in two quite different ways. No matter at what level the tentacle is cut, a proximal cut end always shows one type of behavior, a distal cut end another. Assuming arbitrarily, for convenience, that the activities which characterize the behavior of a distal cut end extend over five millimeters of the length of the tentacle, then we may say that any transverse zone or band of the tentacle, of such length, is capable of these two types of behaviour. When a tentacle is cut so that a certain one of these arbitrarily distinguished zones happens to lie at the distal end of a fragment, the behavior peculiar to a distal end is called forth. But if that same zone of tissue had happened to lie at the proximal end of a fragment, then it would have responded in the very different way which characterizes a proximal end. It readily appears that the position of any given zone of tissue in relation to a cut surface is the occasion which determines whether that tissue shall behave one way, or the other. But when we inquire what is the means by which always the appropriate one of these two alternative responses is called forth, we soon find ourselves at the limit of our present knowledge. However, some suggestions may be made.

The distinctive feature of the response of the distal cut end is contraction. In all probability this is a contraction of more or less specialized muscle elements—the circular muscle-processes

or fibers. Even if it were a contraction of other than specialized muscles, it is nevertheless an expression of the contractility of protoplasm, which for our present purpose amounts to the same thing. As regards the action of these contractile elements, there are two possibilities; they may either be subject to the control of a nervous mechanism, or they may be entirely independent of specialized nervous structures.

If the first one of these two possibilities obtains, then it may be observed that the very definite responses of the tissues situated upon the two opposite sides of a plane of transection imply an equally definite nervous arrangement. We may suppose, for example, that the contractile elements are associated with nerve fibers which extend only distad. In such a case, at least so far as the neuro-muscular mechanism is concerned, transection would be followed by contraction upon the proximal side of the plane of cutting, but not on the distal side. It must be kept in mind that we are now concerned with the contraction of circular fibers only. Contraction of longitudinal fibers appears to play no part in the differences of behavior which characterize proximal and distal ends. Yet in the responses to tactile stimulation we see a similarity to the responses to transection in that, with moderate stimulation, longitudinal contraction takes place only on the proximal side of the point of stimulation. Further, this arrangement would seem to necessitate that the nerve fibers be short—perhaps not longer than the length of the nipple. Otherwise, why should cutting cause maximum contraction of only that narrow band of tissue which forms the nipple and not of the tissue proximal to the nipple? The partial contraction of the tissues proximad of the nipple, as a result of which the fragment of tentacle assumes a conical form, may be a secondary contraction conditioned by the extreme contraction at the distal end and not dependent upon stimulation directly from the cut surface. Or, if we suppose that the contractile elements are innervated indifferently from both proximal and distal directions, then we may imagine that the stimulus of a cut surface is transmitted only proximad, not distad; or else that there is contractile response only to a stimulus from the distal direction—that something inhibits contraction in response to any impulse proceeding from a proximal direction.

If our second possibility shall be found to obtain—that is, if it shall be shown that the tentacle possesses no nervous mechanism of a kind adequate for an explanation of the distinctively different responses of proximal and distal cut ends, then a structural basis for these differences must be sought in the finer details of protoplasmic structure which, with the means now at our command, are still beyond the reach of our senses.

It is essential first of all that we gain full knowledge of the histological conditions of the organism. The peculiar difficulties attending the study of the histology of the actinians, particularly that of the neuro-muscular mechanism, are such that the application of modern methods—see, for example, the work of Havet ('01) -has really advanced our knowledge of the finer details of their structure very little beyond the results of the classic researches of the Hertwigs ('79). If the behavior of the tentacle becomes intelligible on the basis of a nerve-muscle arrangement, then the polarity problem is merely transferred into ontogeny, where we again encounter it in undiminished importance. What determines the origin of that structural polarity which later finds expression in the constantly different behaviors of proximal and distal ends? If no such nerve-muscle arrangement is found, we must then search for some still more recondite protoplasmic mechanism which shall operate in such a way that identical causes -a cut surface- shall give rise to different kinds of effects in the two opposite directions of the length of the tentacle. A tentacle can bend in any direction, and when not specially stimulated it does But when it is stimulated by food it bends directly toward the mouth and in no other way. What is the mechanism at the bottom of this very useful behavior? We may well doubt if that final analysis of protoplasm which is implied in the answers to such questions as these is humanly possible. And so long as the discovery of protoplasmic mechanism appropriate to the final explanation of at least some one vital process remains unachieved, we cannot exclude from our minds the possible alternative—an alternative so remote from our full conception that we may venture to state it only in the form of a question. May it be that a certain protoplasmic structure, no matter how simple or how complex, possessed of a certain definite internal physiological condition, and under a certain definite set of external conditions, namely, the sum of all those physico-chemical factors which immediately affect it, is at any given instant capable of two or more different modes of behavior? This, I believe, is the critical question for our decision between "machine-theory" and "vitalism." Whatever we may think regarding the possibility of a final solution of the question it is only in the continued search for structure within structure, mechanism within mechanism, that we can now see any avenue of approach toward a close-range view of the two alternatives.

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## **EXPLANATION OF PLATES**

#### PLATE I

- Fig. 3 represents a tentacle of Aiptasia. All of the other figures represent tentacles of Condylactis.
- Fig. 1 Stump of tentacle at an early stage of the act of extension, about ten minutes after cutting. Natural size.
- Fig. 2 Four tentacles, two of which have suffered loss of their distal portions. The stumps of the injured tentacles are fully extended, the cut ends being closed by means of the nipples. On the proximal portion of the normal tentacle at the right of the figure, the transverse bands which characterize the entire wall of the tentacle are indicated. Natural size.
- Fig. 3 A very short stump of tentacle, closed and bearing a nipple; viewed in the plane of the surface of the disc. Three times natural size.
- Fig. 4 A tentacle as it appeared three days after a transverse slit was cut in its wall. The wound is at the inside of the sharp bend in the tentacle. Natural size.
- Fig. 5 Fragment from proximal region of a large tentacle, 16 minutes after excision. The distall end bears a nipple. Twice natural size.
  - Fig. 6a The same fragment as in Fig. 5, but about an hour later. Twice natural size.
- Fig. 66 Distal portion of the same tentacle as in Figs. 5 and 6a, one and a third hours after excision from the proximal part of the tentacle, and one hour after the clipping of a very small fragment from its distal tip. The distal cut end bears a nipple. Twice natural size.
- Fig. 7 The fragment represented in Figs. 5 and 6a was fixed in mercuric chlorid 24 hours after excision. This figure shows a view of the proximal end of the fixed fragment. Twice natural size.
- Figs. 8, 9, 10 Pieces of tentacles with proximal end tied to hydrostatic tube. The pieces are distended under the influence of slight internal pressure. The distal ends are nearly or quite closed. Fig. 10 shows an oblique end view of the piece. All twice natural size.

#### PLATE II

All the figures are three times natural size.

- Fig. 11 Piece of Asptassa tentacle with its proximal end tied to hydrostatic tube and under slight internal pressure.
- Figs. 12, 13, 14, 15 Each figure represents an excised tentacle of *Condylactis* transected into two or three pieces. The fragments are shown in their original axial relations to one another. In every fragment the larger end is the proximal end. In Fig. 14, a' represents fragment a with its distal portion in a state of extreme elongation.
  - Fig. 16 An excised tentacle of Aiptasia cut into two pieces; a is the proximal, b the distal portion.
- Fig. 17 An excised tentacle of Aiptasia with its cut pistal end tied to hydrostatic tube, and under 80 mm. internal pressure. The free proximal end is open.



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# A BIOLOGICAL AND CYTOLOGICAL STUDY OF SEX DETERMINATION IN PHYLLOXERANS AND APHIDS

BY

#### T. H. MORGAN

# WITH ONE PLATE AND TWENTY-THREE FIGURES IN TEXT

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# PART I

# INTRODUCTION

The results described in the following pages are the outcome of several years' work. In 1903, when I first began to study the aphids, the possibility that sex is determined by external conditions was the general opinion of zoölogists. The observations of Dageer and Kyber in particular on the aphids seemed to show

that either temperature or food is the sex-determining factor in this group. On this interpretation it might seem possible to regulate sex by controlling the conditions under which the aphids were kept. In the autumn of 1903 Miss. N. M. Stevens and I began our work on the group with these ideas in mind. Stevens undertook the histological examination, while the experimental work was to be done conjointly. The former yielded the results that Miss Stevens published in 1905, but the experimental work gave only negative results. None of the external conditions to which we subjected the rose aphid produced the change from the parthenogenetic to the sexual forms. Later I tried a long series of other experiments in which twigs of the rose with aphids on them were kept in solutions of various salts, magnesium, calcium, potassium, lithium. The solutions, drawn up into the stem by the evaporation from the leaves should be imbibed by the aphids which procure their food by sucking the juices of the plant. These it was hoped might cause a change in the mode of reproduction. The results were negative.

Observations made during the course of the experiments soon convinced us that temperature, at least, is not the cause of the change in the cycle in the rose aphid; for we found that the sexual forms may appear in the late summer before the cool weather has begun. Furthermore late in the autumn parthenogenetic individuals can always be found on the ends of the young twigs of the rose. In one case I kept a potted rose outside of a window and aphids remained on it until December, even after a freezing temperature outside. If brought into the green-house, these terminal aphids may continue to multiply throughout the winter without reproducing sexually. The facts show that temperature need take no direct part in the change in mode of reproduction; they also show that the conclusions that have been drawn from Kyber's experiments are doubtful. The continued parthenogenesis of aphids brought into the greenhouse need not mean that the result is due to removal from the effects of cold, but that those individuals having escaped, so to speak, the influences, whether internal or external, that cause the cyclical change, continue to reproduce by parthenogenesis. Whether, having escaped at the critical period

they could ever subsequently be caused to produce the sexual forms, is not known, but there is nothing that we know opposed to such a view.

Through the courtesy of Prof. W. J. Gies, an analysis was made by Dr. W. Berg of the leaves of the rose and of the maple. The first analysis was made in June when the young aphids had just emerged; the second late in the autumn when the leaves were old and the sexual forms had appeared. There is no very great difference between the two sets of leaves as the accompanying table shows. There is more water in June and more starch in October. The ash is also greater in October. These are the only clues that the analyses furnish as to differences in the food plants. Of course there may be many other substances present that differ in amount not brought out by an analysis of this kind.

	ROSE LEAF				MAPLE LEAF			
•	October, 1905.		June, 1906.		October, 1905.		June, 1906.	
	fresh	dry	fresh	dry	fresh	dry	fresh	dry
Water	60.25		63.39		63.37		72.79	_
Total nitrogen	0.797	2.004	0.971	2.645	0.914	2.495	0.86	3.16
Ether extract	3.09	7.78	2.238	6.114	2.15	5.86	2.10	7.719
Water soluble reducing substances, calculated						!		•
as dextrose		5.33	2.754	7.503	2.364	6.453	1.102	4.050
dextrose, x o.g	6.42	16.15	6.63*	18.06*	4.081	11.14	3.648	13.40
Ash	3.61	9.085	3.069	8.215	4.775	13.10	2.064	7.586

The foregoing discussion is not intended to imply that external conditions are not potent factors in the life cycles of species with alternating sexual and parthenogenetic reproduction. On the contrary, I am prepared to accept such a view despite the negative result of the experiments; but one fact of capital importance has been, I think, overlooked in the interpretation that has been applied to the facts. The results show that whatever the conditions are that bring about the transformation, the change involves the pro-

duction of both sexual forms, the male and the sexual female; hence the conditions do not determine sex in the sense of producing either males or sexual females, but bring to an end parthenogenetic and introduce sexual reproduction. It follows, I think, with probability that we are dealing with two different things here, and that confusion has resulted from supposing them to be the same.

These ideas led me to abandon the hope of finding the clue to sex determination in the external conditions, however important these factors may be in cyclical changes in sex production. In recent years the repetition, by several zoölogists, of the older experiments on tadpoles, caterpillars, etc., that had been accepted as demonstrating the influence of external conditions, has shown with great probability that those experiments did not establish their claim. The discovery at the same time of an internal mechanism associated with sex determination has gradually brought conviction that internal and not external factors determine sex.

In the group of insects, and especially in the group of Hemiptera to which the aphids belong, sex determination has been shown to be associated with an internal factor with which the number or the kind of chromosomes is closely linked. Therefore we should expect, a priori, to find in the aphids some similar factor, if we are to ascribe to it any profound significance.

The most hopeful field for investigation seemed to be in cases where eggs of different sizes exist associated with male and female development. In the phylloxerans, near relatives of the aphids, these conditions are found. The presence in America of many species of Phylloxerans on the hickories gave me the opportunity I sought, and a fortunate discovery of two species, in which the sexual eggs could be obtained in vast numbers, has made the working out of the problem possible, although extremely laborious. My first results were published in 1906; since then I have continued to study the group, but only during the spring and summer of 1907 did I obtain the material that has made it possible to work out, not only the spermatogenesis, but the entire cycle of cytological phenomena. The main facts in regard to the spermatogenesis were made out in October and November, 1907, and the briefest possible statement of the main facts was given before the Society

of Experimental Biology and Medicine, 1908, reserving the complete account until the whole story could be told. The results there published on Phylloxera have been confirmed by von Baehr, 1908, and by Stevens on aphids, 1909. I had also studied the aphids and convinced myself of the identity of the main processes with those of Phylloxerans.

# THE LIFE CYCLE OF THE PHYLLOXERANS

A definite and limited number of generations is passed through by the Phylloxerans of the hickory. In the spring the stem-mother hatches from the winter egg. When the leaves first burst from the bud she attaches herself to the under surface, and piercing the leaf with her proboscis causes the gall-like growth that gradually surrounds her. She soon begins to lay eggs that develop parthenogenetically. These eggs I shall call the stem-mother'seggs. In most species they produce winged individuals, all alike externally, but some carry male eggs only, others female eggs only. This generation I shall refer to as the winged forms, or the second generation. Their eggs I shall call male and female eggs. The male egg is considerably smaller than the female egg, and more of them are present in the adult animal. In most species the winged forms leave the galls to deposit their eggs on the twigs, trunks or leaves of hickory trees. The eggs hatch in about a week. The small eggs produce the minute acrive males (Plate I, Fig. 1), ready to fertilize the females as soon as they hatch; the large eggs produce the sexual females (Plate I, Fig. 3), each containing a single egg, relatively enormous as compared with the size of the female, but in reality no larger than the parthenogenetic eggs of the preceding generations. The female is also sexually ripe very soon after hatching. She is fertilized by a male and deposits her egg on a branch of the tree. From this egg-the winter egg-the stemmother emerges the following spring.

There are certain deviations from this typical cycle which will be referred to in their proper place.

#### SEX DETERMINATION IN PHYLLOXERA FALLAX

This species is found abundantly in the vicinity of New York on young hickories, or on the lower leaves of older trees. When well infected the leaves are covered thickly with the conical galls. There is some question as to the identification of the species owing to differences of observation as to its life cycle. A discussion of this matter is relegated to another section. Here it will suffice to say that in many galls the winged generation is replaced by wingless individuals (Fig. XVIII, A), and these lay the male and female eggs within the gall itself, where they hatch. Owing to this unusual habit it is possible to collect quantities of the sexual eggs and embryos. The formation of the spermatozoon takes place within the male embryo before it hatches. Abundant material for the study of spermatogenesis is therefore readily obtained.

The stem-mother begins to deposit her eggs, one at a time, before the gall reaches full size. The number of eggs is variable, and since the first may hatch, and grow up into an adult which begins to lay, before the stem-mother has completed her series, there may be some overlapping of the two sets of eggs. The number of eggs

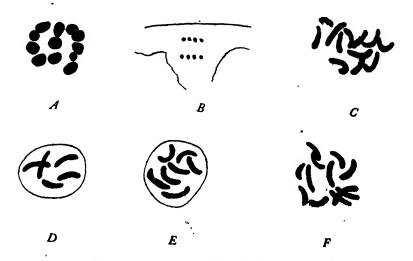


Fig. I P. fallax. A, Equatorial plate of a polar spindle of egg of stem-mother; B, side view of polar spindle; C, chromosomes of somatic cell of embryo from egg of stem-mother; D-E, two sections of nucleus of same; F, another nucleus of same.

laid by the stem-mother varies from one to 60 or more. Different stem-mothers produce different numbers; sometimes sterile individuals are found, sometimes the death of the stem-mother occurs after the gall has begun, in which case its further growth stops.

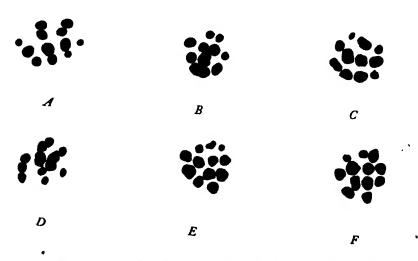


Fig. II A-F, equatorial plates of polar spindle of eggs of winged individuals.

Before the egg is laid the polar spindle is formed. As shown in Fig. I, A, twelve chromosomes of unequal sizes are present. After the egg is laid a single polar body is formed. The nucleus, remaining in the egg, to judge from the chromosomes in its later divisions, contains also twelve chromosomes. This number seems to be characteristic of all the embryos derived from the stem-mother's egg, despite the fact that an important change has probably been initiated already, for some of the embryos produce adults that are male layers; others, female layers. In Fig. I, C-F, the chromosomes of embryos from the stem-mother's eggs are shown. The number seems to be twelve in all cases.

As stated, there are two kinds of individuals of the second generation in P. fallax—the winged and the wingless. Comparison with other species makes probable the view that the wingless forms have been secondarily derived, and replace the winged.

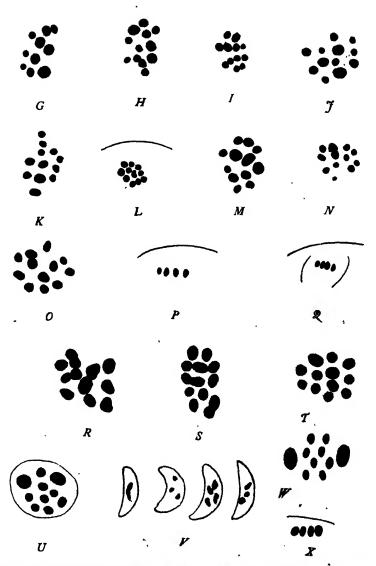


Fig. II a G-O, R-T, equatorial plates of polar spindle of egg of winged individuals; P-Q, side views of same; U, V, W, polar spindles of eggs of wingless individuals; X side view of same.

It is curious to note that the wingless generation bring to maturity one egg (seldom more) at a time, as does the stem-mother, whom, in fact, the wingless forms resemble externally. It has been difficult to get flat views of the polar spindle of the eggs of these wingless individuals. The only two clear cases found are shown in Fig. II a, U and W. Each shows ten chromosomes of which two are noticeably larger than the others and beyond doubt represent a pair of fused chromosomes. I have found eight plates (K-O, R-T) with 12 chromosomes each, and nine plates with 12 chromosomes (Figs. II, and II a A-I) in two winged individuals. All of the winged individuals that I have found—some 300 in number—contained only small eggs and are therefore male-producers.



Fig. III A-D, chromosomes of somatic cells of female embryos.

As soon as the eggs are laid—male or female—a single polar body is given off. It has not been possible to count definitely in this species the number of chromosomes eliminated in the polar body, but the subsequent results show that the outcome must be the same as in P. caryæcaulis where the chromosomes in the polar body can be counted.

After the polar body is given off the female egg still contains twelve chromosomes, as the count of those in the embryonic cells shows (Fig. III, A-D). On the other hand only ten chromosomes are found in the embryonic cells of the male (Fig. IV, A-D). Two have disappeared. In the light of our general knowledge of chromosome behavior two possible explanations of their disappearance may be offered; either in two cases two have fused into

H-K). The accessory chromosomes now begin to contract and clearly move towards one pole, always to that lying in the larger cell. At the same time the chromosomes at the opposite pole fuse or stick together, and the surrounding cytoplasm, retreating to the

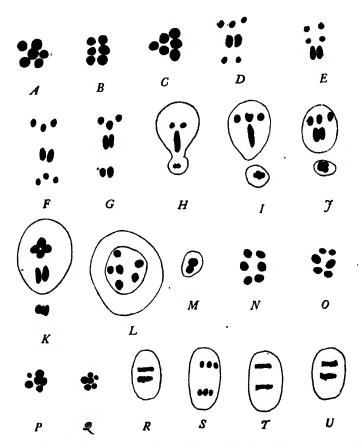


Fig. VI Spermatogenesis: A-C, equatorial plates of first spermatocytes; D-I, dividing first spermatocytes; K-M, after division; N-Q, equatorial plates of second spermatocytes; Q-U, dividing second spermatocytes.

larger cell (as the constriction passes down), there is produced a small cell containing four fused chromosomes, and a large cell containing six, i. e., four separate chromosomes and the two accessories. A nuclear space has appeared around the four chromosomes in the

larger cell even before the accessories have quite approached that region. As they move in, they, with the rest, become enclosed in a common nuclear wall.

This first spermatocyte division is of unusual significance. Hundreds of nuclear stages have been examined, and they all tell clearly the same story. It is certain that the accessories do not divide, but pass entire to one of the two cells formed at the first spermatocyte division. It is true that they are somewhat elongated at one stage, and sometimes give an impression that they are about to divide; but later they contract and pass over entire into the larger cell.

The resting stage following the first division is shown in Fig. VI, L-M. In many cases all six chromosomes can be counted in the larger cells. The small cells come to lie in the corners between the larger cells, or often at the outer wall of the follicle. The chromosomes form a dense mass, staining deeply in iron hæmatoxylin. Their presence in a follicle shows that the cells have passed through the first division—a point not easily made out from the size of the larger cells alone since these, from their mode of origin, necessarily approach closely the size of the prespermatocyte cells.

Equatorial plates of the second spermatocyte are relatively less abundant. Some of them are represented in Fig. VI, N-Q. Four of the chromosomes are sometimes seen to be smaller than the other two—owing to the non-division of the latter. The chromosome plate is more compact, and it is difficult to obtain cases where the chromosomes are distinct.

At the second spermatocyte division all the chromosomes of the larger cells divide equally. There are none that lag behind the rest (Fig. VI, R-U). Two equal cells result, and these become spermatozoa, each carrying six chromosomes.

The small rudimentary cells, present after the first spermatocyte division, do not divide, as far as I have observed, at the time when the large second spermatocyte cells divide. They remain in the follicles as dark bodies which can be identified for some time later when they disappear without producing spermatozoa.

Let us return to the female line. We have seen that the polar

spindle of the female egg contained twelve equal chromosomes. The somatic cells of the female also contain twelve chromosomes. These embryos become sexual females (Plate I, Fig. 1); each carrying a single egg. Before the sexual or winter egg is laid its polar spindle is produced. One clear case that I found showed six chromosomes. Evidently the expected reduction has occurred which from analogy must lead to the extrusion of two polar bodies. In the aphids, in fact, two polar bodies have been found by Blochman and later by Stevens, to be extruded from the winter egg. After the polar body formation, the egg should contain six univalent elements—the same number contributed by the male. Hence the fertilized winter egg will contain twelve chromosomes, corresponding to the number found in the parthenogenetic eggs.

With the preceding facts as a basis, we can sum up the chromosomal history of P. fallax as follows, beginning with the spermatozoon.

The functional spermatozoon contains two accessory or sex chromosomes and four others. The unfertilized sexual egg contains likewise two chromosomes, the homologues of the accessory chromosomes, and four others. The fertilized eggs contain, therefore, four accessory chromosomes and eight others. Consequently the stem-mother contains these twelve chromosomes, all of which appear in the polar spindle of her egg. One polar body is extruded, all twelve chromosomes dividing, twelve go out and twelve remain in the eggs. The eggs are destined to become female-producing and male-producing individuals; both sets of individuals, as well as their ripe eggs containing twelve chromosomes. The female eggs are larger than the male eggs. Each gives off a single polar body. In the female all twelve chromosomes presumably divide equally—twelve going out, twelve remaining in the egg. In the male egg, on the contrary, two of the accessories are presumably given off with the polar body, ten chromosomes remaining in the egg. The two entire chromosomes given off at this time must be the partners of the accessories; hence we can understand how in the male two of the chromosomes have no partners, and therefore cannot pair during synapsis. They pass over in the first division to one pole at the time when the other paired chromosomes separate.

This occurs at the first spermatocyte division. In the second spermatocyte division the larger cell containing six chromosomes divides, each chromosome dividing. Two spermatids result.

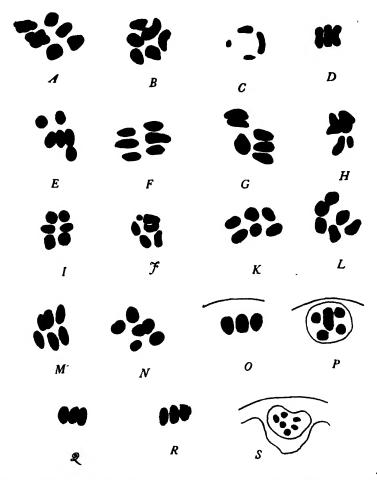


Fig. VII Phylloxera caryaccaulis. A, B, E-N, P, S, equatorial plate of eggs of stem-mother; C, polar body of same egg; D, O, Q, R, side views of polar spindle.

The small cells, containing only four chromosomes, do not divide, and later degenerate.

In the sexual female there is presumably a synapsis period, the twelve chromosomes pairing to give six bivalents. Two polar bodies are given off, one chromosomal division being equatorial, the other reductional, in the ordinary sense, i.e., the reduced number of chromosomes appears; so that two of the sex chromosomes pass into one of the polar bodies; their partners remaining in the egg. The egg having two accessories gets two more accessories from the functional spermatozoon which accounts for the four accessories that our analysis calls for.

## SEX DETERMINATION IN PHYLLOXERA CARYÆCAULIS

The life cycle in this species is typical of the group. The chromosomal history is as follows: The polar spindle of the stem-

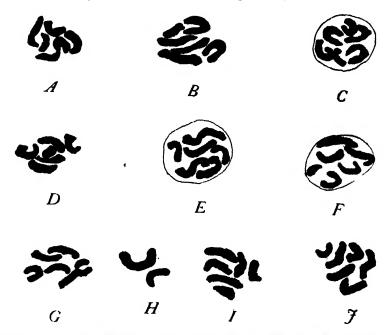


Fig. VIII A-J, chromosomes of embryo from stem-mother's egg. H, shows only two chromosomes, for size relations.

mother's egg contains six chromosomes of nearly equal size (Fig. VII, A, B, E-N, P, S). These eggs give off a single polar body (Fig. VII, C).

The cells of the embryos contain six chromosomes, as shown in Fig. VIII, A-f. It is a point of some importance to know

whether the chromosome group is the same for the female-producing and for the male-producing embryo. The observations leave the point in doubt since no differences were found, but the number of cases was not large.

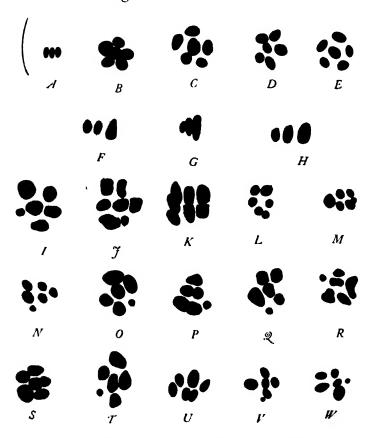


Fig. IX B-E, equatorial plates of polar spindle of female egg; A, side view of same; I-W, equatorial plates of polar spindle of male eggs; F-H, side views of same.

The female-producing winged form of the next generation contains a few large female eggs. The polar spindle in these eggs contains a group of six nearly equal chromosomes as seen in Fig. IX, A-E. In the polar spindle of the mate egg of other winged individuals there are also six chromosomes, but of very unequal sizes (Fig. IX, I-W). There is one largest chromosome, almost twice

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as big as the medium-sized ones, and one smallest much smaller than the medium ones. Evidently some change has occurred which, without altering the number of the chromosomes, has given them different sizes. The meaning of this change will be apparent later.

A single polar body is given off from the female and from the male eggs. I have studied the polar body formation in this species with great care and have tried for a long time to obtain demonstrative telophase stages. The accumulative evidence is convincing, I think, although despite the long labor that the search has involved I have not yet found a case in which in the telophase both daughter plates could be counted except for one female egg.

In this female egg, as shown in Fig. X, J, the chromosomes divided equally, six going into the polar body and six remaining in the egg.

In the male egg I have found no case in which both daughter plates can be counted, but in one case the outer plate showed six distinct chromosomes. Polar bodies show in the best cases at least six chromosomes as seen in Fig. X, C-I.

The number of chromosomes left in the egg can be determined by the number found in the embryonic cells. Here a curious fact comes to light. In some male embryos all the cells contain five chromosomes of nearly equal sizes—one can often be seen to be larger than the rest-and the others can be sorted into two pairs (Fig. XI). In other male eggs there are six chromosomes—one of them being much smaller than the others, and generally, though not always, connected with one end of another chromosome (Fig. A comparison of size-relations shows that the smallest chromosome here corresponds to the smallest in the polar spindle of the male egg. It would seem to be absent in the other—the fivetype—but facts to be developed later show beyond doubt that it is not absent, but united to one of the other chromosomes. Thus in the cells of the male embryo the chromosomal number is six. the same number counted in the females. The result seems at first incompatible with that for the other species in which the male contains two less chromosomes than the female. In reality the results agree; for, here the true female number will be shown to be eight,

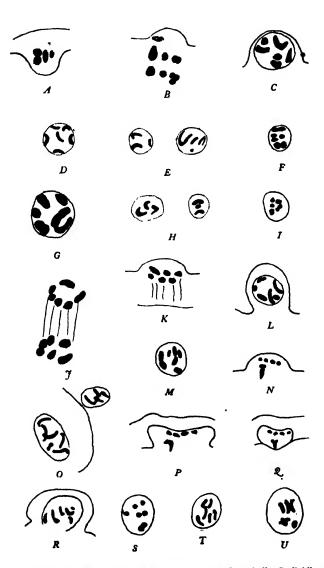


Fig. X A-I, polar spindle and polar body of male egg; A, polar spindle; B, dividing polar plate; C-I, polar body; J-O, polar spindle and polar body female egg; J, telophase; K, outer pole of another telophase, inner pole lost; L-N, polar body; O, polar body and female pronucleus with six chromosomes; P-U, polar bodies of eggs, sex of egg not determined.

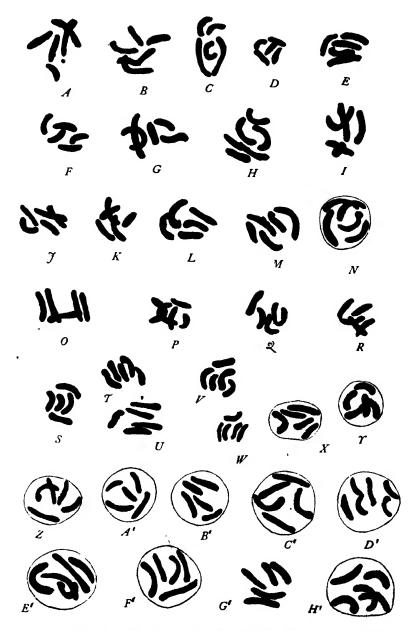


Fig. XI Chromosomes of somatic cells of male embryo—five chromosome type. In many cases the cells are from the same embryo.

not six as stated, although in the embryos from female eggs the number of chromosomes counted is six (Figs. XIII and XIII, A). The spermatogonia of P. caryæcaulis also show either five nearly

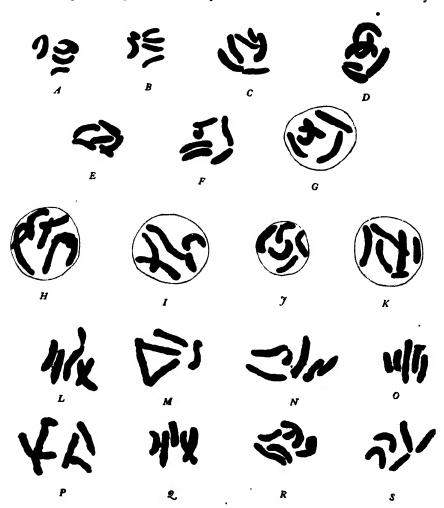


Fig. XII Chromosomes of somatic cells of male embryos; six chromosome type.

equal chromosomes, or five such and one very small one. The spermatocytes show an equatorial plate made up of three equal or nearly equal-sized chromosomes (Fig. XIV, C), or else of three

equal or nearly equal-sized chromosomes and one small one (Figs. XV, N-R; XVI, S, T). These two types obviously correspond to the two somatic types, and here, as there, all the cells of one embryo are alike in that they fall into one group or the other.

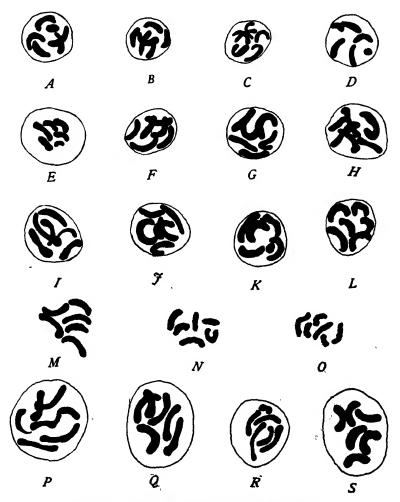


Fig. XIII Chromosomes of somatic cells of female embryos.

At the beginning of the division two of the chromosomes show a clear line in the middle where the halves will subsequently sepa-

rate (Fig. XIV, F). The other large chromosome shows no division at this time (Fig. XIV, F), nor does the smallest one, if it can be detected (Fig. XV, S). As the two pairs separate the

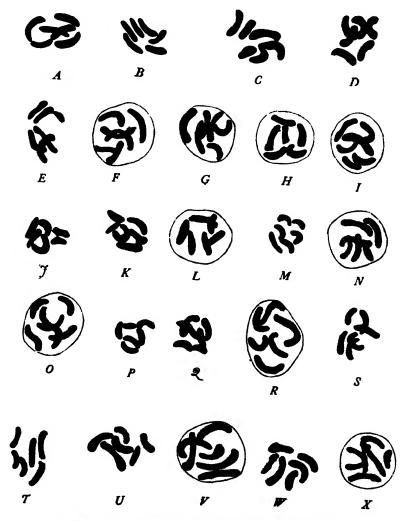


Fig. XIIIa Chromosomes of somatic cells of female embryos.

larger chromosome becomes drawn out into a somewhat dumbbell shaped mass (Fig. XIV, D). At this stage it is very noticeable

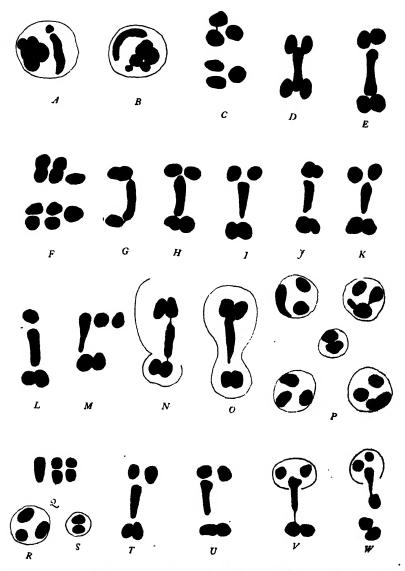


Fig. XIV A, B, nuclei just prior to the formation of the first spermatocyte, several chromosomes bunched together, two separate—late synapsis; C, two equatorial plates; D-W, first spermatocyte divisions.

that the two chromosomes that have not divided—the lagging or sex chromosomes—almost invariably fuse into a single chromosome; or if they do not fuse, become so closely apposed that no line of separation is visible (Fig. XIV, G-M). Moreover this union is not due to the optical effect of superposition but to actual contact.¹ When I first studied the spermatogenesis of this species I believed for some time that there was but a single lagging chromosome.

As the cytoplasm begins to constrict it soon shows that one cell is to become larger than the other (Fig. XIV, O; Fig. XV, G; Fig. XVI, C). The lagging chromosome—the larger visible one—becomes greatly elongated extending to each daughter plate. It is often enlarged at both ends and narrower in the middle, as though it would divide into two (Figs. XIV, D; XVI, D, F); yet such is not the case, for a little later it shortens, thickens, and retreats towards the larger cell. In its behavior it resembles, on the one hand, the aphids as closely as it does P. fallax on the other.

During the final stages of division the smaller cell loses nearly all of its cytoplasm (Figs. XV, L, X; XVI, X. Y, Z); and when finally separated consists of two closely apposed chromosomes with only a thin covering of cytoplasm (Fig. XV, L; Fig. XVI, Z, A'). The larger cell shows a nuclear space around the two chromosomes before the lagging chromosome has reached the same level (Fig. XIV, V; Fig. XV, G; Fig. XVI, P, Q R); but a little later its end becomes included in the clear area. At this time, in some cases, one may detect the smaller lagging chromosome budding out, as it were, from the end of the other lagging chromosome in the larger cell (Fig. XV, G; Fig. XVI, F-7). Its free end becomes round, and it now assumes in such cases an independent position in the new nucleus (Fig. XV, L, N, O; Fig. XVI, C-I). Very often it remains attached by a thread or band to the other accessory. In some cases the two remain closely united, the smaller like a knot on the side of the larger.

In the second type, the small lagging chromosome does not emerge from the other at this time, and only three nearly equal

<sup>&</sup>lt;sup>1</sup> In a few cases, perhaps abnormal, I have detected the amallest chromosome lying next to the other accessory, Fig. XVIIa.

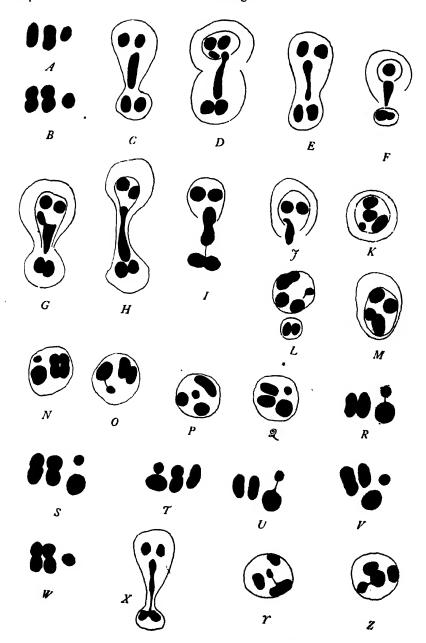


Fig. XV First spermatocyte division stages.

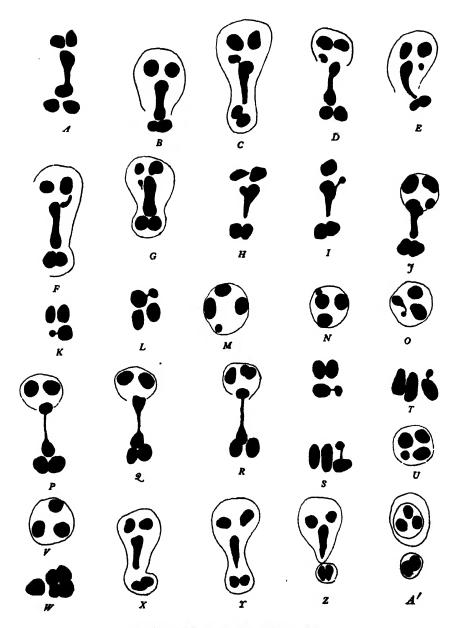


Fig. XVI First spermatocyte division stages.

chromosomes are present—the largest one being therefore the double accessory (Fig. XIV, U-W; Fig. XVI, P,Q). This difference in the behavior of the small accessory has made the study of this species very perplexing. It might appear that we are dealing here with two species, one with three and the other with four chromosomes, but this view is negatived by a number of facts. A close examination of the sexual males fails to reveal two types, and since the sexual individuals of different species are readily determined, the evidence is strongly in favor of one species. On the tree, at New Bedford, Mass., on which this species was found swarming out of the galls only one kind of gall was present, and since I have studied the sexual forms of the commoner galls in this region it is improbable that two species here exist. The fact that all intermediate types between union and separation of the chromosomes are found also indicates that they all belong to the same species; in some individuals the two chromosomes are always separate, in others united by a connecting thread; in others the smaller is stuck to the side of the larger, and others show only a single larger chromosome. On the assumption that there are four chromosomes in the male, the results can be brought into harmony with the facts found for the stem-mother's egg, and for the number of chromosomes present in the spindle of the male egg. This conclusion also harmonizes with the results on the other species, P. fallax.

In the resting stage following the first division, the nuclei of the larger cells contain three or four chromosomes each, and it is interesting to note that the type that prevails in each individual corresponds with the type found in the stage preceding division as is shown when both stages coexist in the same testis.

The equatorial plates of the second spermatocytes also show one or the other type (Fig. XVII, L, N, O); but the chromosomes are crowded and these stages are difficult to find. I have not studied them extensively. As the chromosomes divide it is apparent that there is no lagging chromosome (Fig. XVII, D', E', F) and a few successful cases show clearly that the three or four chromosomes, according to the type, divide and pass to the poles. These are the two cells from which the spermatozoa develop.

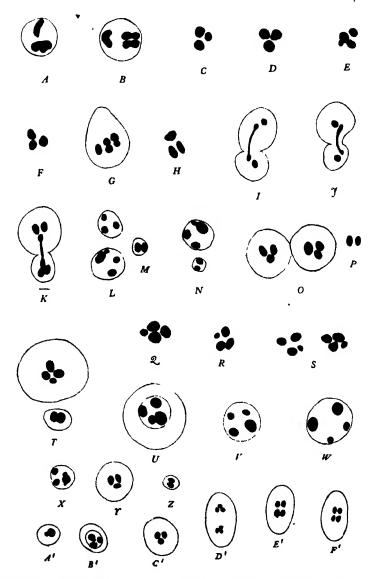


Fig. XVII A-B, nuclei just prior to the formation of the first spermatocyte; C-H, equatorial plates of first spermatocytes; I-O, division of same; P, rudimentary cell; Q-W, nuclei prior to second spermatocyte; X, Y, B', C', equatorial plates of second spermatocytes; Z, A', rudimentary cells; D', E', F', division of second spermatocytes.

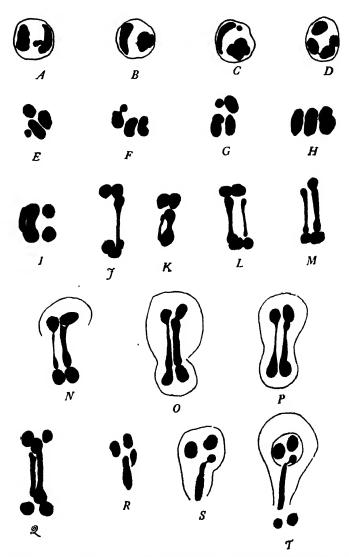


Fig. XVIIa A-D, nuclei prior to first spermatocytes; E-G, equatorial plate of first spermatocytes; H-T, side views of dividing first spermatocytes. In K, L, M, N, O, P, Q, the second lagging chromosome is separate from the first.

The rudimentary cells of the first spermatocytes, containing two chromosomes (Fig. XVII, M, P), can be found wedged in between the other cells, or lying at the periphery of the follicle. They do not appear to divide and disappear later.

In a few individuals, apparently somewhat abnormal the two accessories are distinctly separated. Several cases of this sort are brought together in Fig. XVIIa.

Returning to the female egg, where six nearly equal chromosomes were counted, it was shown that the six divide so that six pass out and six remain in the egg. The somatic cells of the female embryo also contain six equal or nearly equal chromosomes, differing from the six-type of the male in that one chromosome in the latter is much smaller than the rest. Each female produces a single egg. I have been unable as yet to obtain the polar spindle of the egg, but just before it develops the chromosomes appear in the nucleus as a group of deeply staining bodies in a less deeply staining plasmasome. In these cases I have counted four chromosomes, including one small one. The evidence from this source is not entirely convincing, but, if corroborated, it adds further evidence in favor of the view that eight, not six is the diploid number.

My interpretation of the chromosomal history of this species is, then, as follows:

The functional spermatozoon contains four chromosomes, two ordinary ones and two lagging chromosomes of which one is large and one very small. The two lagging chromosomes show a tendency in many individuals to form a single body producing the appearance of only three chromosomes for the reduced number. The sexual egg contains the same number of chromosomes as the sepermatocyte, i. e., two ordinary and two sex chromosomes, the two latter possibly united. After fertilization eight chromosomes are present but only six appear since the two pairs of sex chromosomes unite. These six chromosomes are nearly the same size. Hence each smallest must be fused with its larger partner (accessory). This same number, and in the same combinations, persists in the polar spindle of the stem-mother's egg. Here all must be divided equally since the same number, six, is found in the

embryos. These embryos become female- and male-producers the winged migrants—whose eggs are large or small. The former contain six chromosomes in the polar spindle of nearly equal size which seem to represent the same combinations existing in the preceding generation. In the male spindle a difference appears, there being one largest, four intermediate and one smallest chromosome. The only way in which such a combination can be accounted for, on the assumption that here too the original number is still retained—as in P. fallax—is that the two smallest of the eight have shifted over from their larger partners and have united with each other; the larger partners have also combined to produce the very large chromosome. The "purpose" of the shifting is a preparation for the polar division in which one of the larger chromosomes and one of the smallest passes entirely out of the male egg to leave six chromosomes in the egg.<sup>2</sup> These six often appear as five, owing to the subsequent union of the largest and the smallest sex chromosomes. Hence we find in the spermatogenesis two pairs of ordinary chromosomes that unite during synapsis and two sex chromosomes that have no pairs. These, in consequence, pass over to one pole at the first spermatocyte division that represents the "reduction division." The theoretical questions connected with these results will be discussed later.

## PRECOCIOUS SEXUAL DIFFERENCES CORRELATED WITH MALE AND FEMALE EGGS

The relations described in the preceding section throw an important light on the differences in size of the male and female eggs. It is obvious that the difference is connected with the precocious development of the sexual individuals and not with the condition of maleness or femaleness of the egg, as such. In other animals size differences may also exist, the female being much larger than the male, yet the eggs are all of the same size. The differences in adult size in the latter case result from differences in the rate or time of growth after hatching. It is therefore of unusual interest

<sup>&</sup>lt;sup>2</sup> In P. fallax the same result is probably shown when ten chromosomes appear in the polar spindle, two being of greater size.

to note that in two other species of animals in which a difference in size exists between the male and the female egg, the males are practically sexually mature when they emerge from the egg. I refer to Hydatina senta and Dinophilus apatris. In both of these the males are sexually mature a few hours after emerging from the egg, and without growing larger to any extent they become sexually potent. The relation has, I believe, heretofore entirely escaped the attention of zoölogists.<sup>3</sup> Does it give us any clue to sex-determination?

The first point to settle is whether the male egg is smaller than the female because the young are sexually mature at the time of hatching, or whether the relation is the reverse, the male being smaller because it comes from the small egg.

The female emerging from the egg is as near sexual maturity as the male, hence it is not apparent why the difference in size should depend on the maturity of either sex; yet comparison with other animals shows that only where early sexual maturity exists are the eggs of two sizes. All of the materials that make the sexually mature female must exist in the egg, since there is neither time nor opportunity to get them after hatching. The female body is larger than that of the male, and the egg is larger. The difference in size therefore of the male and female is owing to the fulfillment of those conditions necessary for producing the sexually mature female directly from the egg and the same holds for the male. In other animals, sexually dimorphic, the conditions that determine sex may also exist in the fertilized eggs, but while the factor that determines the size differences must also be present in potentia, it need not be one that makes a difference in the egg size. It follows, I think, with great probability that the difference in the size of the male and female eggs in Phylloxerans can be referred to the difference in the sizes of the males and females that these eggs produce. In other words the two kinds of individuals and their sex is already determined before the egg is laid and before its polar spindle has developed. Therefore sex is determined in the presence of all of the chromosomes.

<sup>&</sup>lt;sup>2</sup> See Morgan, T. H., for a brief statement of this relation. Exp. Zoöl.

There can be no doubt that the two kinds of eggs are not simply the result of the accidental amount of material contained in them. If this were the case, there would be no sharp line of demarcation but a gradual series of sizes, and if the result were purely chance, there would be more eggs of intermediate sizes than of large and small. The facts are the reverse. There are two sharply separated groups of eggs and while, of course, there are fluctuating size differences, these center around two widely separated modes.

Two alternative views offer themselves at this point. Either there is a sex-determining factor in the eggs of the stem-mother that separates female-producing from male-producing individuals, or in the life of the winged individuals some factor external or internal turns the scale in one or in the other direction. Let us dwell for a moment on these possibilities.

If a sex-determining factor is present we have found no clue to such in our study of the chromosomes. The eggs of the stemmother are all of a size. If events subsequent to the egg formation of the stem-mother determine whether the individual produces male or female eggs, the following considerations should receive attention. Foremost is the observed fact that the male producers contain small eggs, but more of them, and the sum total of egg-material may be actually greater in some male producers than in other female producers. If then some condition should bring about the setting free of a larger number of eggs from the ovary the presence of so many developing at once might turn the scale so that the alternative of the smaller egg is followed. If this view seems too improbable it might be imagined that the food conditions are less favorable at first than later on, or vice versa, and in consequence the first individuals that mature would be male producers, the later ones female producers. I find no facts to support this view. One argument far outweighs these possibilities. The male egg shows from its very earliest embryonic development that it is characteristically a male and I do not think, in the light of other facts regarding the influence of the chromosomes on the early development of the egg, that the results here can be safely

<sup>4</sup> I refer to the early differences in the cytoplasmic relations of male and female embryos.

ascribed to the absence of two chromosomes. For these and for other reasons I am inclined to think that the determination of the male and female producers is not the result of chance or of some external factor but that a mechanism, perhaps, or something definite at least, within the stem-mother's egg determines the characters of the result.

If one were inclined to make fine distinctions it might be said that we are dealing here with two independent, yet correlated phenomena. The size differences in the egg are connected with the production of small individuals and of large individuals. The sex of these individuals is determined by other factors; the two events are so correlated that they coincide, but I doubt the advantages of such an explanation.

## IS THE ELIMINATION OF THE SEX CHROMOSOMES FORTUITOUS OR DETERMINATIVE?

Are the two chromosomes thrown out of the male egg different from their partners that remain behind? Are the homologous chromosomes of both of these pairs in the female line identical or qualitatively different? Are the two left in the sexual egg identical with the two in the male, or are they so to speak complementary in their relation to sex determination? An answer to these questions involves several assumptions concerning the rôle, qualitative or quantitative, of the chromosomes in sex determination.

If we assume that the chromosomes thrown out of the male egg are female-producing (and we are forced to this view if we assume that there are two kinds of sex chromosomes) it follows that the functional spermatozoon is the bearer of the male determinant, yet it clearly corresponds to the "female producing" spermatozoon of other insects. Following the same line of thought, the sexual egg should eliminate its male-producing chromosomes—otherwise after fertilization the egg would contain only "male-producing" chromosomes. Fertilization would bring together the male-producing chromosomes of the spermatozoon and the female-producing chromosomes of the egg; the result being a female whose sex on the hypothesis is due to the egg and not to the "female

producing" sperm. Here we meet with one of the many paradoxes to which the view, that the sex chromosomes are sexually different, leads.

On the contrary assumption that the male egg eliminates the male chromosomes we leave unexplained the essential point of what then determines that that egg is to become a male. Other combinations of the sex chromosomes are conceivable, but all lead alike to contradiction or difficulties on the assumption that the chromosomes are really differentials and that their elimination from the egg and sperm is discriminative.

The opposite point of view looks upon the two pairs of chromosomes of the female and the single pair remaining in the male egg as strictly equivalent, pair for pair. As long as either member of a pair is eliminated from the male egg the essential conditions for male production are fulfilled. The union of the homologous members of the pair in Phylloxera caryæcaulis would mean that this is a step preparatory to their separation at the next division. The pairs might be turned either way on the spindle since it would be a matter of indifference which member of the pair was eliminated and which remained in the egg. The separation would be fortuitous. The difference between male and sexual female would be a quantitative difference so far as the chromosomes are concerned. But since the total number of chromosomes is the same in the parthenogenetic and the sexual females some other condition must determine the transition from the one to the other. What that other condition could be unless synapsis we do not know any more than why in the body the cells are differentiated in many directions without any chromosomal differences to distinguish them.

If the transition from a parthenogenetic female to a sexual female can occur without chromosomal diminution—and the transition involves as vast a number of differences as those that distinguish male from female—may not the same sort of change take place in the case of the transition from the parthenogenetic to the male form? We have seen, in fact, that a change has taken place a generation before the male egg appears that is prophetic of the change that comes later. May not this change be the real condi-

tion that determines sex? The chromosomal elimination being the consequence and not the cause of sex. Such interpretation would involve a profound alteration in our views of the relation of sex and chromosomes. It would mean, for one thing, that in ordinary forms there are other differences in the two kinds of sperm that are male and female producing than that of the number of chromosomes. The female spermatozoon would not be female producing only because it contains one or two more chromosomes than the male, but, conversely, it contains these chromosomes because other changes have already been initiated that cause the accessory chromosome to move into that cell. Just as in the male egg the behavior of the sex chromosomes is discriminative—two entire chromosomes going into the polar body, so in the sperm two entire chromosomes go into the "female-producing" sperm. It seems to me the evidence may mean that there is a mechanism in the cell that is determinative in regard to sex factors; and that this mechanism has come to have associated with it particular chromosomes.

The chromosomal relations, however, remain still a fact and a very extraordinary fact. It is hard to conceive that these relations have no connection with sex determination, even if we grant that changes take place before the differences in the number of the chromosomes occur that foreshadow the later sex differences. Why this extraordinary behavior in regard to the chromosomes if it is not concerned with the sex relation? It may be as injudicious to ignore the hehavior of the chromosomes, as to deny that antecedent events also connected with sex determination are operative. evidence that we have at present seems to point to the conclusion that in the insects at least, the chromosomes are involved in the series of changes that determine sex; but may not the chromosomes be only a part of the process that leads to sex determination? The visibility of the chromosomal changes has caught our eye; the obscurity of the antecedent changes has caused them to be ignored; but in such a case as this one where a parthenogenetic cycle is introduced, the analysis shows that conditions antecede the chromosomal elimination—conditions also essential to sex determination.

Such considerations show at least that there are questions connected with sex determination that may be as important as the visible differences relating to the quantitative factor At present however our evidence is largely confined to the recognizable quantitative changes in the chromosomes. A problem of exceptional interest arises in connection with the oögenesis of the parthenogenetic eggs that produce parthenogenetic individuals (the stem-mother's oögenesis for example) and of the parthenogenetic eggs that produce the male and sexual female individuals. Is there a synapsis of the chromosomes? Even though the full number of chromosomes is retained, this fact does not preclude the possibility of synapsis. I have studied this problem with some care but the decision is so fraught with difficulties that I have decided for the present to withhold any attempt to answer the question until I can give the matter fuller consideration.

## THE LIFE-CYCLE OF PHYLLOXERA FALLAX

The doubts that have arisen in regard to the identification of this species (see Pergande) are due to an incomplete knowledge of its life-cycle. Only a series of consecutive observations made at short intervals could reveal the relations of the successive generations. During two years I have made such observations and at the critical periods have preserved quantities of material every three or four days. The facts discovered in the course of these observations show how difficult it would be to unravel the lifecycle without a very complete series of stages; for, the inhabitants of galls first formed may pass through certain phases before the inhabitants of later formed galls reach those stages. There is thus an overlapping of stages. Second, the number of individuals of a phase in the cycle is closely connected with the size of the gall, which in turn is generally dependent on the time at which the gall is produced. Third, the production of winged and wingless forms by the stem-mother is not the same for all galls.

The following data give the results of observations made during the spring of 1908 on material collected on the Palisades north of Fort Lee, New Jersey. On May 23 young galls, many not yet closed in, were found. Each contained one stem-mother and from 1 to 83 eggs. The number of eggs is almost directly proportional to the size of the gall. Thus in small galls the number of eggs was 3, 1, 4, 3, 3, 6, 4. 5, 0, 0, 0, 0, 4, 9, 1, 0, 2, 2, Somewhat larger galls contained 11, 13, 16, 6, 6, 8, Medium-sized galls contained 14, 18, 39, 29, 23, 35, 27, 33, 38, 30, 37, 21, 47, 32, 45, 37, 36, 29, 15, 43, 39, 44, 27, 36, 19, 23. Big galls contained 25, 30, 30, 54, 36, 47, 42, 67, 62, 74, 31, 83, 36, 46, 22. In the largest galls a few of the eggs had hatched.

Several days later (June 4) a considerable advance had been made. The chief events may be here summarized. Many of the eggs laid by the stem-mother had hatched. She was still present, however, and continued to lay eggs. The young that first hatched had become either wingless or winged individuals, or both in the same gall. The relation of these two kinds of individuals to each other is interesting and will be referred to later. The most important fact brought out by a study of the galls of this date (166 galls in all) is that there is a sudden break in the series of offspring; the eggs first laid producing individuals that grow to full-sized winged or wingless individuals, while those laid later produce a different kind of individual that never grows larger and remains throughout life the same size as when first hatched. These individuals I have named the supernumerary or dwarf females. Equally interesting is the discovery, that in the larger galls as many as 46 eggs may produce the large individuals, and then the smaller series abruptly begins; while in the smallest galls only one to three or four or more large individuals are produced when the small series begins. There seems to be here not a predetermined number of large and dwarf females, but the conditions of life determine when the one kind ceases to be produced and the other kind begins. The two types of individuals must, however, be predetermined by alternative possibilities possessed by each egg.

The supernumerary or dwarf females differ from their large wingless sister-forms, and from the young of the latter in a number of points (Fig. XVIII). The shape of the body is entirely different and resembles that of the sexual male; but it differs from

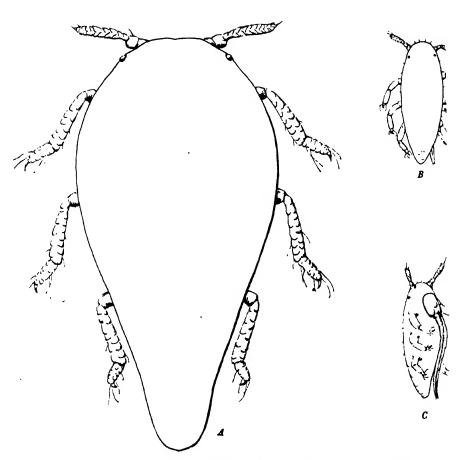


Fig. XVIII P. fallax. A, wingless individual of second generation; B-C, rudimentary females; B, dorsal; C, ventral view.

the male in two important respects; first, the dwarf individuals have a very long proboscis which in this species is absent in the male; second, there are no testes within the abdomen as in the males, where they form a relatively enormous mass. Otherwise the dwarfs are so similar in external form to the sexual males that their true nature was uncertain until they were studied in serial sections. These showed the absence of the testes and the presence of rudimentary ovaries and ducts resembling those of immature parthenogenetic females. There was nothing to indicate that the dwarfs could become sexual females. In fact the latter contain each an enormous egg when they hatch.

The dwarfs can at once be distinguished from the young of the same generation by the length of the proboscis. In the dwarfs the proboscis is as long as the body, while in the young it is not more than two-thirds as long. The presence of the well-developed proboscis might lead one to infer an exceptional ability for prolonged life, and this might readily suggest that these minute individuals are predestined to short circuit the life-cycle by remaining on the tree to produce the sexual forms of the following year. While I cannot positively deny to them such an existence, the facts that I have indicate on the contrary that these dwarfs are destined to a brief existence, and die without progeny.

In the following table, I, (June 4) I give the results of an examination of the contents of 166 galls. In the first column is given the number of the gall; in the second, the size of the gall; in the third, the total number of individuals; in the fourth, the number of large wingless or winged individuals. For very young stages it is not easy to detect the wings, but for older stages the presence of rudimentary (or of complete) wings is readily seen. When the wings are spread, i. e., when the insect is an imago, I have underscored the figures indicating the number of individuals. The other columns include the number of eggs and the number of dwarfs or supernumerary females.

TABLE I

NO. OF GALL	SIZE	Total	Large Wingless	Winged	Dwarfs	NO. OF GALL	SIZE	Total	Large Wingless	Winged	Dwarfs
I	medium+	25	12			44	small +	15	5		
2	medium+	53	4			45	small +	9	١		
3	medium	28	4			46	small +	2			
4	medium	15				47	small +	9	2		
5	medium	16	2			48	small +	19			
6	medium	32	8			49	small +	15	7		r
7	medium	118	4	19		50	small +	13	9		
8	medium	21				51	small	4			
9	medium	23	10	į		52	medium	12		1	
10	small	10			1	53	medium	19			
11	small	13	ī	į		54		11	4		2
12	small	49	4			55		13	4		2
13	medium	22	13		İ	56	small	10	4		
14	medium	27	8			57	1	3	1		
15	medium	41				<b>5</b> 8	.,	11		1	
16	medium	35				59	small	6		1	
17	medium	12	7			60	small	6			
18	medium	11				61	small	4	į	ĺ	
19	medium medium	13			į	62	very small	2			
20	medium		1 2	11	į	63 64	very small	7	l		
22	medium	59		11		65	small	12		}	
23	medium	49 17	4	Ī	į	66	small	5	-	ĺ	
24	medium	51	6	1	1	67	small	7		1	
25	medium	17		1		68	small	32 16	10		
26	medium	18	3 4			69	small	15	6	İ	
27	medium	10	2			70	small	23	8	1	
28		10	4	ļ		71	large	208	34	12	
29	medium —	25	8	i	6	72	large	63	34		3
30	medium —	9	4	1	r	73	large	64	11		4
31	medium -	24	6	- 1	2	74	large	11	5		1
32	medium —	17	5		_	75	large	36	3		3
33	medium —	13	6		2	76	large	40	14	1	1
34	small	4	2	l	1	77	large	52	14		
35	small	8	6		1	78	large	49	25		2
36	small	7		- [		79	large	67	18		1
37	small	6		I	i	80	large	49			
38		26		}	2	81	large	25			2
39	small	12			2	82	small	13			2
40	small	41		1		83	small	10		- 1	
41	small +	20				84	small	13			2
42	small +	29			-	85	small	13	_		
43	small +	12				86	emall	14	-		

TABLE I-Continued.

NO. OF GALL	SIZE	Total	Large Wingless	Winged	Dwarfs	NO. OF GALL	SIZE	Total	Large Wingless	Winged	Dwarfs
87	small	14			1	128	small	5			
88		9			2	129	small	4			
89	very small	9				130	small	3			
90	very small	30			3	131	small	29	2		1
91	big	38	-		2	132	medium	14			ĺ
92	big	53				133	medium	34			
93	big	35	10			134	medium	21			
94		79	-		'	135	small	9	:		
96		40	-			136	small	7			
97	medium	13	3		1	137	medium	22			
98	medium	23			2 ,	138	*medium	27			
99	medium	9			1	139	medium	26	4		2
100	small	6			'	140	medium	33	i		1
101	small	4	, ,			141	medium	16			
102	small	7				142	medium	25			
103	small	7				143	medium	13	!		
104	small	4			1	144	medium	87	8	18	<b>[</b>
105	small	7				145	small	. 5			
106	small	3			1	146	small	8			i
107	medium	35	7		1	147	small	24			
108	medium	20	5		1	148	small	28			
109	medium	12	3		2	140	big	75	10	9	
110	medium	12	4		1	150	small	9			
111	medium	10				151	medium	79	4	11	
112	medium —	39	_		2	152	medium	83	5	8	
113	medium —	14	6		1	153	medium	74	4	12	
114	medium —	10	0		1	154	medium	72	6	2	3
115	medium +	60	i i	7		155	medium	44	2	7	
116	med um +	116	8			156	medium	79	4	12	
117	medium +	126	7		3	157	small (?)	28	5		
118	small	6			1	158	small (?)	42	4	4	
119	medium	72	7			159	small (?)	165	9	1	3
120		28	3		2	160	small (?)	56	5	8	
121	small	9	2			161	small (?)	44	4		
122	smail	14	3		1	162	small (?)	61	4		4
123	small	2				163	small (?)	40	5		2
124	small	5			1	164	size (?)	52	15		4
125	small	11	2			165	size (?)	62	17		6
126	small	3				166	size (?)	44	13		1
127	small	13				-					

<sup>\*</sup> In No. 138 two stem-mothers were present.

The table shows a number of important details not mentioned above. The minimum and maximum number of eggs laid by the stem-mother can be approximately estimated from the data. The extremes vary between 1 (in No. 20) and 60 (in No. 115).5 It is true that still larger numbers are found in some other cases, but in most of these the wingless individuals of the second generation were already full sized and may have begun to layeggs. In fact in some cases, especially in the last ones given, this is certainly true, since large and small eggs were present. The smallest numbers also may not represent the entire output, since many of these galls were still small and later would no doubt contain more eggs of the stem-mother. Nevertheless since in some of these cases the second generation, consisting of a few individuals, contained fully grown insects, it is probable that the complete number of eggs had been laid. This conclusion is confirmed when we find, as in No. 106, a dwarf female was present and only two other individuals.

Another interesting point is also brought out. In No. 165 there were 17 large individuals (or young ones that would have become larger) and 6 dwarfs. This means approximately that the first 17 eggs developed into large individuals, and the 18th was a dwarf. It follows that there is no fixed number of large productive individuals but the conditions determine the result.

The conclusion is of some general importance since it shows that external conditions may be responsible for producing not a graded series of differences, but a sudden change, in other words, a mutation. The eggs have therefore a dual capacity, external conditions determining which alternative is realized.

The largest number of large individuals, including wingless and

<sup>&</sup>lt;sup>6</sup>Several measurements were made of the three kinds of parthenogenetic eggs. These gave the relative sizes of the eggs.

TEM-MOTHER EGGS	MALE EGGS	FENALE EG	Gŧ
16 × 9	16 × 9	20 × 10	
17 × 9	18 × 9	20 X 11	
14 × 8	17 × 8	20 × 91	
15 × 8			
15 × 8			

<sup>&</sup>lt;sup>5</sup>As many as 83 were recorded in another count: (See ante).

winged, is found in No. 71, where 46 individuals are present. It is probable that even this number does not represent the maximum, as all those capable of growth may not have been at this time full grown; but this number is probably near the upper limit, and there can be no doubt that the lower limit is far below this.

In the tables, cross lines in the first columns separate galls from different leaves. There is, in a general way, a resemblance between the contents of galls from the same leaf, indicating that similar conditions give similar results. While this holds in general there can be no doubt that the later arriving stem-mothers producing small galls are differently affected. In this connection the occurrence of winged individuals presents some curious results. They occur most frequently on the same leaf, i. e., some leaves contain them, others are nearly free from them. Two interpretations are possible; either special conditions call forth this form or else in certain strains the production of winged individuals is more potent (still possibly subject to external conditions). The method of breeding in this species may appear to lend some probability to the latter interpretation. All the males and females from a single gall and from the same or from neighboring leaves will remain on those branches from which the leaves arise, except for the few winged migrants, and even these seem at times to deposit within the gall. In the following year their offspring will be likely to crawl out to the new leaves on that branch, hence at least we might expect them to appear in groups. The production of winged forms occurs also in aphids where in some species in almost every generation a certain number of winged migrants appear. Whether here also they owe their presence to special external conditions or to an internal mechanism has not as yet been definitely determined.7

A few minor observations may be here noted. In only two cases have I ever found more than one stem-mother in the same gall. In both of these cases two stem-mothers occurred. Such cases are probably due to two stem-mothers occupying exactly the same position—possibly to a late mother entering a still open gall. When one recalls that the entire leaf may be completely

<sup>&</sup>lt;sup>7</sup> See Morgan, "Experimental Zoölogy," pages 322-334.

covered with galls—converted into a mass of galls in some cases—it is surprising that two stem-mothers do not oftener produce a common gall. When the galls are near together they crowd each other and flatten at the contact planes, but I have never found any communication between them.

The size of the gall does not appear to depend on the presence of the second generation; for, in one case I found a large gall occupied by a single stem-mother without progeny. She must have been enfeebled or parasitized, yet the gall was fully developed. On the other hand if the stem-mother dies after the gall has begun and before she lays her first eggs, the gall ceases to grow. Experiments should be made to see if the gall will develop after the stemmother is killed and only her early progeny left in the gall. The transfer of stem-mothers of different galls would also be profitable and might give interesting "hybrid galls."

The data indicate, if they do not prove, that the fertility of the stem-mother is intimately connected with the growth of the gall, and the growth of the gall in turn seems to depend on the condition of the leaf when it first begins to develop. The earliest galls develop rapidly and the stem-mothers in them lay the maximum number of eggs. Later galls remain small and the stem-mothers produce very few eggs. That the result is due to the condition of the leaf when the gall is first formed and not to the time of year is shown by an observation I made on this species in 1906. The buds on a certain branch were delayed as much as two weeks behind the others. When they did develop they became infested with P. fallax. The stem-mothers must therefore have waited two weeks for the bud to unfold yet the gall became full size. The converse point of view, that small galls are produced by weak mothers that will lay few eggs, may hold for some cases, but in general the facts indicate that it is the condition of the plant rather than that of the animals that determines the fertility of the latter.

Contrary to the behavior of other species of phylloxerans, the winged individuals of P. fallax may deposit their eggs within the gall. This is indicated by the empty abdomen of some of the winged individuals. That others may emerge and deposit outside seems also not improbable. This in fact would be the only

means of dispersal for the species at present.\* The depositing within the gall may be connected with the condition of the gall that fails to open soon enough to let the migrant escape. In fact the galls do not become well opened until the sexual males and females are ready to crawl out. This divergence from the traditions of the genus might seem to throw some light on the occurrence of the apterous females of the second generation in P. fallax. The late opening of the gall may have led to the depositing of eggs within it by some of the winged generation. The presence of wings became therefore superfluous, at least except for colonization on other plants; hence the opportunity was given for the appearance of apterous females that would better insure the continuation of the race, since they are not exposed to the great dangers of failure to find a suitable plant, etc. Teleological argument of this kind, is, I think, of no special value. It is shot through and through by the kind of anthropomorphism that exposes much of our zoölogical work to reproach. For, to go no further, it is equally manifest that the same advantage would accrue to other species even if they early opened their galls.

That the appearance of the apterous forms was due to a sudden change—a mutation—is more than probable, since both winged and wingless exist at present without intermediates and since the wingless condition is obviously an innovation. But the wingless individual is not simply a curtailed winged individual—lacking the wing-factor, in the latest phraseology—is shown by her egg-laying habits. In the winged forms all the eggs ripen at the same time; at least they all become fully formed before any are laid. In the wingless forms, on the contrary, the eggs ripen one (or two) at a time in rapid succession and are at once deposited—an apparent adaptation since the place of deposit is at hand and taken advantage of, while the winged forms must retain their egg for deposition in a different location.

A point of unusual biological significance is found in the fate of the supernumerary eggs of the stem-mother. She may lay, as we have seen, as many as fifty or more; yet the maximum number

<sup>&</sup>lt;sup>8</sup> In the present case, however, the winged migrants contained only male eggs. This would lead to crossing but not to dispersal in any other sense.

of those that become full size is not more than half this number. Although, potentially, of course, each egg must be looked upon as having the possibility, its fate is determined by the condition of nourishment of the stem-mother. A few of the later laid eggs produce the dwarfs, the rest begin to develop but do not hatch; they may be found in older galls, turned black and on the road to decay, an astonishing fact, in itself, possibly connected with the enormous productivity of their sisters that mature—a point discussed later.

There is an interval of four days between the observations recorded in the last table and those given in the next table, II, (June 8). In this interval a large number of sexual oggs have been laid in the large galls. It is noticeable that the number of supernumerary females has increased very little, indicating that the later laid eggs of the stem-mother do not hatch.

It will be seen that only eight winged individuals were found in these thirty-two galls. The galls were still closed so that there can be no question of their escape. The wingless individuals must be very prolific, as shown by the number of sexual eggs found in some of the galls where few wingless forms are recorded. Some deduction must be made, however, for small eggs recorded as male eggs, since these are mixed with stem-mother eggs still present. The size of the male eggs and of the stem-mother eggs is so nearly the same that their record is here combined.

Even at this time the very small galls produced by late arrivals contain very few eggs or immature individuals (in addition to the stem-mother, not recorded in the count). In one case (No. 200) there was present in a very small gall only an immature stemmother. In another case (No. 174) there was present one immature apterous form, three eggs and one dwarf, showing that the latter must have developed from a stem-mother's egg, since the second generation was not yet mature. Other similar cases have been found.

<sup>&</sup>lt;sup>9</sup> In the table "total," third column includes immature individuals and stem-mother eggs. Male and female eggs, seventh and eighth columns, also include whatever stem-mother eggs have not yet hatched. Such eggs would fall into the "male" column which is therefore too great. The stem-mother is not counted in with the total.

It is not without interest to note the proportion of male and female eggs at this time, but it would be unsafe to make any deductions, since the numbers are as yet too small and the records confused owing to the presence of stem-mother eggs. It is nevertheless worth observing that even in the small and very small galls, where conditions have been extremely adverse, female eggs occur without any relative diminution in their number.

TABLE II.

NO. OF	SIZE	TOTAL	LARGE WING- LESS	WINGED	DWARFS	MALE EGGS	FEMALI EGGS
167	large	5	13	1			
168	medium	193	2	;	5	135	51
169	medium	112	5		5	67	35
170	medium	114	4	!	2	73	35
171	medium	81	. 3	ļ	3	50	24
172	medium	52	4	5		38	5
173	small	14	' 3			_	
174	small	5	1		1		
175	small	20	4		2		
176	small		3	;	2	9	2
177	small	20	2	i	2	15	2
178	small	26	4	1	2	16	3
179	small	1	' 3		2	11	1
180	small	9	2	i I		6	-
181	small	25	3		1	16	5
182	very small	8	1		2	4	
183	very small				1	•	
184	very small	•	. 2	į	1	11	1
185	very small	1	1	1	1	••	-
186	email	, ,	2	Ì	-	7	-
187	very small.		-	1	17	,	5
188	small	5			2	12	_
189	medium	-3	2		_		7
	small	59	5		3	39	12
190	small	4		1	2	8	i _
191		15	2	İ		-	8
192	small	15	3		1	4	
193	small	16	2		-	9	4
194	small	7	1		I	4 6	1
195	small	12	I		3	0	2
196	very small	3					1
197	very small	3					
198	small	14	1		1	9	3
199	medium		2		2	32	7
200	very small	StM					

Table III, June 17 (201-240) brings out not much that is new but confirms some of the findings of the preceding data. Despite the fact that male and sexual females have now begun to hatch (in some of the later cases these are reckoned in with male and female eggs) the number of dwarfs has not materially increased. Male eggs preponderate somewhat, the sum total being 342 female eggs to 454 male eggs.

## THE IDENTIFICATION OF THE SPECIES OF P. FALLAX

Confusion has arisen as to the identity of this species owing to the variations in its life-cycle. Walsh, who first described the species, states that he never found a winged individual in the galls, although he has opened hundreds of them. Pergande's observations on this gall extending over several years "run counter to those of Walsh, for I have found this particular gall swarming with the winged female." Pergande accounts for this discrepancy on the ground that his own observations were made from early May until June, and those of Walsh from June 17th to the end of the month "when the winged forms had already forsaken the galls." Pergande continues: "If the galls be opened early in May, or before the nipple has opened, they will be found filled with winged insects, pupæ, numerous eggs and what appear to be larvæ. These supposed larvæ, however, upon careful examination are not larvæ hatched from eggs deposited by the stem-mother, but the true sexual individuals, both males and females, produced from eggs deposited freely by the winged females."

My own observations furnish results that harmonize in part the observations of Walsh and of Pergande. They show that many galls do not ever produce winged individuals and that their place is taken by apterous forms; also that in some galls both types are present but as yet I have found none containing only winged individuals. The history of the galls was so closely followed that it is quite certain that the winged forms did not first appear and then leave the galls. On the other hand when winged forms occur their number is not large and the galls could not be said to be filled with them. It follows, in all probability, that in different

TABLE III.

NO. OF GALL	SIZE	TOTAL	LARGE WING- LESS	WINGED	DWARFS	MALE EGG8	FEWALE EGG8	sex Q	sex ō
201	small	13	2		1	5	5		
202	small	16	1		4	5	4	1	
203	small	22	2	i	6	7	7		
204	small	44	4		4	30	6		
205	small	6	2	2					
206	small	•	3	2					
207	small	8	4	-	4		Ì		
208	very small			1				-	
209	very small		1	}		2	2	1	
210	very small		1	-	1	6	1	1	
211	very small	-	2			14	6	1	
212	very small		2	1	1	15			
213	large	30	0	6	15	23		13	
214	medium	18	2			9	5	2	
215	medium—		1	1	2	1	4	2	1
216	small +		7	1		4	7		4
217	small +		3			14	28	1	2
218	small +	16	2	1	į i	15	9	1	
219	small +	24	3	;		13	3	j	5
220	small	13	3	1					
221	small	16	1 3	1	I	6	6		
222	small	38	3	}		27	8		
223	medium	31	2	!	1	16	9	2	1
224	medium	27	10	2	4		01		1
225	small	15	1			6	5	2	I
226	small	25	2	1		10	13		
227		7	I			2	2		2
228	b:g		12			44	70		
229	big		11			32	19	35	8
230	big	100	6			55	39		
231	big	111	17			80	89		
232		172	3			9	1		
233		9		6					
234		9	}	4		1	1 1	-	
235			1		]	12	4		3
236_		3	1	1		23	27		
237		5	1		1	39	11		
238	_	6				35	11		
239		4				12	27		
240		3	1	1		22	24		

localities the proportion of wingless and winged forms varies widely. Local races or local conditions may be the cause of this variation, and near New York and near Stamford, Conn., the cycle is intermediate between that described by Walsh and that given by Pergande.

### LIFE-CYCLE IN PHYLLOXERA CARYÆCAULIS

As stated on a preceding page the life-cycle of this species is typical. When the gall is mature it cracks open and allows the winged individuals to escape. These fly out and those that alight on the leaves deposit their eggs on the under surface of the leaves along the midrib, especially near the base. Hundreds of eggs, large and small, may be found on the same leaf. A count of eggs from the leaves showed large female eggs and small male eggs approximately in the proportion of one to five. The data obtained in this way are open to some objection, because if the male layers should escape from the galls first, there would be at first a disproportionately high male rate. Or if the male layers were more active fliers, they might scatter more widely and more of them fail to reach the leaves of the trees from which they came. Again if the males hatch sooner than the females, proportionately too few male eggs would be left on the leaves. For perfectly accurate data the counts should be made throughout the migration period and this I have been unable to do. Nevertheless I think the proportions given above represent an approximately true estimate.

Of far greater importance are the counts of the male producing and female producing inhabitants of each gall, as these are all the offspring of the same stem-mother, herself the product of a winter egg, fertilized by a "female producing" spermatozoon. The galls had been placed in Carnoy solution and preserved in 95 per cent alcohol. They were opened and their inmates placed in absolute alcohol and then in xylol, and examined under the microscope.

<sup>10</sup> Equal parts of absolute alcohol, glacial acetic acid and chloroform, saturated with corrosive sub-

The abdomen and its eggs become so transparent in the xylol that the size of the contained eggs is readily made out.

Most of the galls were closed except a few at the beginning and end of the series, as indicated by the small number of migrants present. Even in these open galls a few immature individuals still remain. In the galls in which a large number of winged forms are recorded the number of immature individuals is small.

The 34 galls recorded in Table IV contained 4824 male producers and 1704 female producers (migrants) or 2.8 to 1. There is much variation as to the number of eggs contained in each individual; the female-producers as a group containing fewer than the male-producers. If we estimate the male producers as averaging 16 eggs and the female producers 8 eggs, the proportion will be 77,184 male eggs to 13,632 female eggs, or 5.6 to 1. The excess of males is therefore again shown both by the presence of more male producers containing more eggs, as well as by the less exact estimate based on the supposed average number of eggs.

TABLE IV.

MALE PRO- DUCERS	FEMALE PRODUCERS	IMMATURE		MALE PRO- DUCERS	PEMALE PRODUCERS	IMMATURE ;	
9	1	9	open	32	1	many	
8	24	54	opeň	106	2	_	
8	11	8	open	23	8		
120	1		closed	41	13	13	open
13	331	-		16	5	10	open
152	177			7	0	3	open
240	0	_		38	5	90	open
229	0	295		36	3	63	open
185	0	92		209	11	53	opening
219	29	- 1		278	0	56	closed
87	209			111	106	199	opening
361	0	1		3	386	24	
127	0	-		383	4	31	closed
390	14	-		•	328	11	closed
323	3	_		40	0	197	closed
240	0	_		335	27	27	closed
291	0	- 1		293	8	21	closed

The more important facts brought out by a study of the inhabitants of these galls remains to be discussed. Some few galls contain only male producers, and there is one record at least where all the migrants were female producers. The great majority of the galls contained both sorts of migrants, with a preponderance of male producers. The results have a rather unique bearing on the question of sex-determination as will be pointed out later.

Two additional facts of prime importance must be also noted I kept a record of the size of the galls. No relation between the proportion of male to female producers and the size of the gall was observed. In nearly all of the galls a few or even many very small individuals may be found. These carry a very few eggs, but the eggs are full size of their kind. I have observed cases where only a single female egg wes present. There can be little doubt that lack of food is the cause of the small size of these individuals. Their greater abundance amongst the later hatched individuals supports this interpretation as well as other facts observed. It follows, therefore, that food conditions do not determine whether a migrant becomes a male or a female producer. It is probable, therefore, that internal factors have already determined the result. Whether these internal factors can be traced to internal or to external conditions in the preceding generation is not evident from the facts given, but there is another point that would seem to indicate that the factors are internal in the first generation also. The stem-mother continues to lay her eggs up to the time when the gall opens. Before this event when the gall is swarming with larvæ and the walls completely lined by them, the conditions for obtaining food must be less favorable than during the early stages of the gall. Yet both amongst the first eggs laid, and amongst the last laid, male and female producers occur. It is difficult to see how food conditions determine the result; for even if the eggs should be affected only at a certain period on which the momentary condition of nourishment might act still we should expect a greater preponderance of one or of the other sex, according to whether the general conditions are favorable or the reverse.

The data furnish strong evidence in favor of some sort of

internal factor, that preponderates especially in those cases where a large number of eggs of one kind are produced. These numbers are so large that the results cannot be due to chance. The nature of the factor involved will be discussed later.

### THE SEXUAL FORMS OF OTHER SPECIES

The sexual forms of only a few of our Phylloxerans have ever been seen. Pergande describes 4 out of a total of 33 species. The minute size of the sexual individuals, as well as the fact that the eggs from which they come are often deposited on the limbs or trunk of the tree where they are difficult to detect accounts in part for this lack in our knowledge. By the following simple method, however, it is very easy to obtain the third generation. The galls should be collected when the winged migrants are ready to emerge and placed in a glass dish covered with fine cloth. The water from the galls may collect on the glass and drown the migrants unless special precautions are taken. I use large flat dishes and turn them upside down so that the galls lie in the middle of the cloth. At other times I have put the winged individuals into paste-board boxes, covered with cloth. The eggs are deposited on the glass, cloth, galls or paste-board, as the case may be. In the course of about a week they hatch; the small, numerous males will be found running rapidly about, while the females move more slowly.

Having found that the sexual forms in P. fallax and P. caryæcaulis are mature on hatching, I was led to hope that mature sexual forms of all the other species might also be obtained, by the method given above. To my surprise it was found that in three other species studied all the eggs laid by the migrants were the same size—not of two sizes as in the other two species. Thinking that I might have obtained eggs of only one sex, I opened large numbers of individuals and still found eggs of only one size. I then made sections of the individuals hatched in my dishes and found that the sexual organs were entirely immature. Furthermore, careful study of the individuals showed that they are all alike, or so similar that I detected no differences by means of which they could be separated into males and females.

A microscopic study of the reproductive organs showed that these were so immature that I was unable to tell whether the individual would be male, sexual female, or parthenogenetic.

In only a few cases as stated above have the sexual forms of the American species of Phylloxerans been described. This is to be regretted because it leaves our knowledge of the life-cycle incomplete at a critical point, and also because the sexual forms are more different in different species than are the parthenogenetic individuals, and therefore are of the utmost importance in the classification and identification of this difficult group. By means of the method here described one may obtain an abundance of material that would be in some cases very difficult to find under natural conditions.

I have given figures in Plate I of the sexual forms male and female of the two species dealt with at length in this paper, and also of the sexual (?) forms of three other species found in the vicinity of Woods Hole, Mass. In the latter only one form exists, as I have stated. Sections show that the reproductive organs are in a rudimentary stage and while I have not made very careful study of them, still I have not been able to distinguish the male from the female organ at this time. This fact is all the more striking when it is recalled that in the other species the male organ forms a conspicuous feature even of the early development and the large testes of the just hatched male contain ripe sperm. In the female, too, the egg at the time of hatching almost completely fills the body and is so conspicuous that it can be seen through the transparent wall of the body.

It remains for the future to discover the fate of these sexual (?) forms of the other species. The long proboscis may indicate that they live on the bark of the trees. If so their small size would make it difficult to detect them. I enclosed in a cloth bag a branch of a tree and placed within it many ripe galls. A subsequent examination of the branches failed to reveal any Phylloxerans, but the conditions were not very favorable.

The idea suggests itself to any one familiar with the facts here recorded that the third generation of these species is not a sexual generation at all, but one destined to continue the race by partheno-

genesis another year. It is not inconceivable that they produce a different gall another year, but as yet there are no facts to support this view.

The sexual males and females of P. fallax are shown in Plate I, Figs. 1-4, the first two drawn from life, the second two from preserved specimens. The male shown in Fig. 1 was probably slightly flattened, while held under the cover slip, so that the preserved specimen, Fig. 3, gives a truer idea of the form.

The sexual male and female of P. caryæcaulis are shown in Figs. 5 and 6. The difference in size is less marked than in the last case.

The remaining three figures 7, 8, 9, of the Plate, represent the single type of individual that emerges from the eggs of the winged migrant of three species. The first of these comes from a large, elongated stem-gall, quite abundant in Woods Hole. The gall, closed at first, cracks open to set free its migrants from whose eggs this form hatches. The species appears to be P. subelliptica, Shriner; but Shriner's description is very incomplete. Pergande who has seen only the gall questions whether it may not be a form of P. caryæcaulis. I had in fact at first confused these two at Woods Hole, but an examination of P. carvæcaulis from Woods Hole showed migrants with two kinds of eggs as in the New Bedford type, while the migrants of the present gall contain only one kind of egg. Obviously they are different species, or else different phases of the same cycle. The form shown in Fig. 7 is entirely different from the sexual forms of P. caryæcaulis. It is not uninteresting to find that the polar plates of the eggs of the migrants of this form show six chromosomes, (Figs. XIX, A-F) as in P. carvæcaulis, but these are not of different sizes.

In Plate I, Fig. 8, is shown the individual that emerges from the egg of the migrant of a species which I provisionally identify as P. caryæglobuli, Walsh. Pergande cites the descriptions of Walsh and of Shriner, but questions whether Shriner's account does not apply to another species. Walsh's description is too brief for accurate identification. The sexual (?) form here described is so specifically marked that in future there should be no difficulty in settling the status of this species, if the plan of hatching the eggs of the winged migrant, here proposed, is followed.

The last figure, Plate I, Fig. 9, is from a small leaf gall with a short nipple opening beneath. When open, four or five short bracts border the margin. The galls seem to corresp ond to P. depressa, but again the description is too imperfect to make identification certain.

These figures show, I hope, the value of this stage in the life cycle for systematic identification. Until this stage has been further studied in more species, and until their relation to the sexual forms is known, our knowledge of the genus must remain, as at present, most fragmentary.

In connection with the study of the sexual (?) forms of these species I have also studied the chromosomes of the equatorial plates of the migrants in the hope that a knowledge of the chromosome number might help in disentangling the systematic identification of the species, but also and primarily in the hope of discovering whether two types of chromosome groups exist where only one sexual (?) form has been discovered. The first question can be answered decisively; the second, with less certainty. The chromosome groups are sufficiently different to aid materially in identification. Within these species I have not been able to distinguish two types of polar spindle plates as in P. caryæcaulis and occasionally in P. fallax, but the number of individuals found with perfectly distinct groups of chromosomes has not been large (despite the great amount of labor expended in finding the polar spindles) so that I should not like to speak positively on this point.

Polar plates of the polar spindle of the eggs of P. caryæglobulare shown in Fig. XIX, G, H. Twenty-two rounded chromosomes of very different sizes have been counted in several plates. As I pointed out in 1906, it is interesting to find so large a number of chromosomes within the genus in which the winged individuals are so closely similar. Evidently the number, as such, is of no significance.

In P. depressa there are six chromosomes (Fig. XIX, I, J) four large and two small, as determined by a polar spindle of the stemmother's egg and by a late nuclear stage of the same sort of egg. In a species whose sexual form I have not yet obtained, P. caryæfoliæ, the polar plates show eight chromosomes of different sizes

(Fig. XIX, Q, R, S). Whether the size differences that appear are significant I cannot state as yet. The somatic cells of embryos from these eggs (artificially obtained) show also eight chromosomes.

In P. globosum, the polar plates contain six chromosomes (Fig. XIX, K-N) but in this case they are not rounded but elongated. Their bent form renders a study of their size relations difficult.

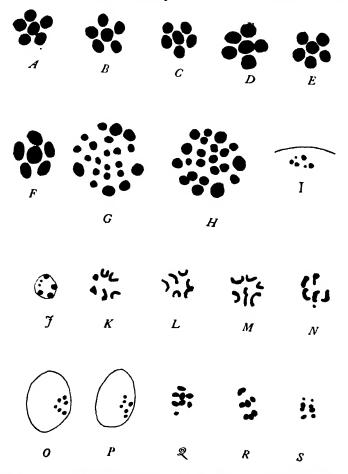


Fig. XIX A-F, equatorial plates of polar spindles of egg of migrant of P. subelliptica; G, H, ditto of caryæglobuli; I, equatorial plate of polar spindle of egg of stem-mother of P. depressa; J, late egg-nucleus of same; K, L, M, equatorial plate of polar spindles of egg of migrant of P. globosum; N, equatorial plates of polar spindle of stem-mother's egg of same; O, P, late egg-nucleus of same; Q, R, S, equatorial plate of polar spindle of egg of migrant of P. caryæfoliæ. None of the figures drawn to scale.

Six chromosomes are found in polar spindles of P. subelliptica much like the female group of P. caryæcaulis (Fig. XIX A-F). The preceding facts and figures show that identification of

The preceding facts and figures show that identification of species may be materially assisted by a knowledge of the chromosomes. It may turn out that the failure to discover two kinds of groups of chromosomes in the eggs of the migrants has a real meaning in connection with the presence of only one form in these galls.

# SPERMATOGENESIS IN APHIDS

Stevens described in 1904, in the first spermatocyte division in aphids, a lagging (accessory) chromosome, which, instead of passing to one pole, divides equally so that each of the first spermatocytes receives a half. All of the chromosomes were found to divide again equally in the second division to produce, in all, four spermatozoa. The result stood in the way of any attempt to bring the aphids into line with other insects possessing an accessory and made difficult any conclusion regarding the relation of the accessory to sex production. Stevens overlooked two essential facts in the spermatogenesis, namely, the fact that the lagging chromosome does not divide, as she had supposed, in the first division; and second, the fact that one of the products of the first spermatocyte division is much smaller than the other and subsequently degenerates.

During the winter of 1907–1908 I discovered in the phylloxerans the two points just noted. These facts I communicated to Miss Stevens, and she most generously gave me some of her former preparations of aphids to study. These with others of my own, that I had made some years before, convinced me that while the failure of the lagging chromosome to divide is more difficult to make out, the aphids show in all essential respects the same relations as do the phylloxerans. I urged this conclusion on Miss Stevens, who, after renewed investigation of her material, came to the same conclusion. Her results were sent to me in May, 1908, for publication in the Journal of Experimental Zoölogy, but owing to delay in publication did not appear until January, 1909. Meanwhile von Baehr, who had been working on aphids, published conclusions in 1908 similar to those found by Stevens and myself.

It is not my wish to enter here into a detailed account of the spermatogenesis in aphids, since Stevens has already gone over the ground, but I should like to state a few of the results of my own observations that will serve to form the basis of comparison between the spermatogenesis of the aphids and that of the phylloxerans. In one not unimportant point I get a somewhat different impression of the facts from that given by Stevens in her last paper, namely, that the process, by which the division of the lagging chromosome takes place, while variable, is more regular than Stevens' account might lead one to infer. That irregularities occur, that whole cysts may "go wrong," as in other insects, is not to be denied; but in the great majority of cases the lagging chromosome moves into the large cell from which the two spermatozoa are ultimately formed (after the second division).

I have chosen of the several species of aphids examined three species from the Willow, for description. Presumably the first of these is the same species studied by Stevens who did not identify the species she used. Mine were obtained abundantly at Woods Hole, Mass.; those of Stevens, at Harpswell, Maine.

In Aphis salicola the spermatogonia contain 5 chromosomes beyond any doubt (Fig. XX, A-C). These can best be counted at a stage just before the equatorial plate is produced.

Three nearly equal chromosomes appear in the equatorial plates of the first spermatocyte (Fig. XX, D-F). At the first spermatocyte division two of the chromosomes divide, the third is drawn out (Fig. XX, G-M). Later stages in the division are shown in Fig. XX, M-X. Here, as in the phylloxerans, the lagging chromosome is not withdrawn into the larger cell until the very last moment, when in fact the nuclear sac has begun to appear around the chromosomes. The final stages in the process seem to involve a flowing or contraction of the chromosome, whose end in the large cell gradually increases in size as the chromosome shortens. In the final stages the lagging chromosome is often surrounded by only a thin film of protoplasm, so that the two cells—the larger and the smaller—appear to be connected by a very delicate bridge. This bridge is found at times when the end of the lagging chromosome, still within the small cell, is slightly enlarged. The bridge

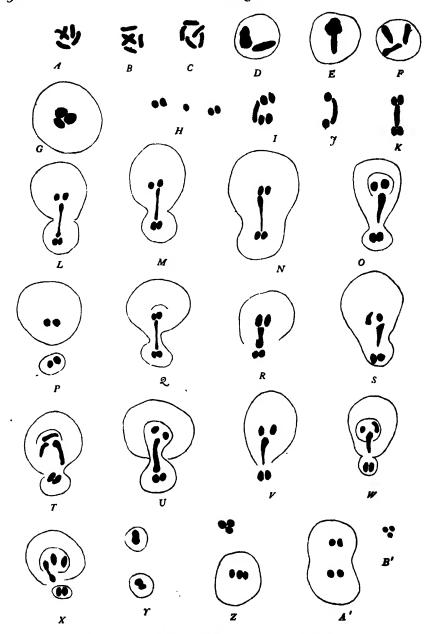


Fig. XX Aphis salicola. A-C, spermatogonial nuclei; D-F, stages just prior to first spermatocytes; G, equatorial plate of first spermatocyte; H-X, stages in division of first spermatocytes; T, rudimentary cells; T, equatorial plates of second division; T, second spermatocyte division, T, after division.

bulges sometimes to one side. That artificial conditions, such as handling or osmosis, might break such a delicate connection at this time is not at all improbable, and such an artificial result might give the impression that the accessory is actually divided. Moreover if the bridge arches toward or away from the observer, the effect may be produced at certain focal levels of discontinuity between the ends of the lagging chromosome, when none such The arch containing the connecting thread of the accessory might also be cut off by the knife, and lie in another section, where its extreme tenuity might defy discovery. Such considerations make me sceptical as to whether the lagging chromosome in this species of aphid ever normally divides into two. Figures like that of Plate I, Fig. 3, in Stevens' paper in which two nearly equal cells contain each one end of the accessory do not seem to me to represent accurately the conditions that I have seen, if on no other ground than in the absence of a bridge of cytoplasm around the connecting thread of the accessory.

In the willow aphid the rudimentary cell is extremely small and contains very little cytoplasm. It does not subsequently divide and slowly degenerates.

The equatorial plate of the second spermatocyte division of the large cells shows three chromosomes (Fig. XX, Z). These divide equally to produce each two spermatids (Fig. XX, A'). In all essential respects the results agree with those that I have found in the phylloxerans.

Von Baehr's facts on a willow aphid A. saliceti with the same number of chromosomes agree with those given here. He also determined that while the female parthenogenetic egg contains six chromosomes, the male egg, after extrusion of its polar bodies, contains only five. He leaves open, of course, as I had done also, the question as to how the number becomes reduced to five. The most plausible inference was obviously that one was lost in the polar body. Stevens' earlier statement (1905) that "there is no evidence in my material of any difference between the maturation of the female parthenogenetic egg and that of the male" is probably incorrect, and her count of the same number of chromosomes in the somatic cells of the male and female embryo must have been an error.

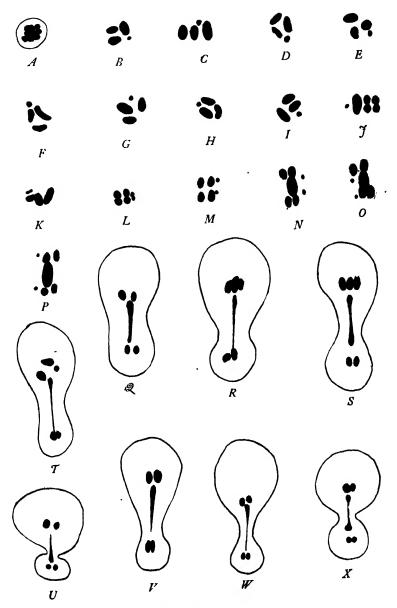


Fig. XXI Lachnus dentatus. A, stage prior to first spermatocyte; B-I, equatorial plates of first spermatocytes; J-X, division stages of same.

In 1907 Tannreuther published an account of the germ cells of an aphid, Melanoxanthus salicis. He has confused the sequence of the stages to such an extent, and has overlooked so many of the essential facts, that a detailed criticism of his statements would be unprofitable. I suspect furthermore that his account of intranuclear polar body formation is entirely erroneous.

Another species of aphid, Lachnus dentatus, gives rise to sexual forms late in the autumn. It contains a large accessory and another

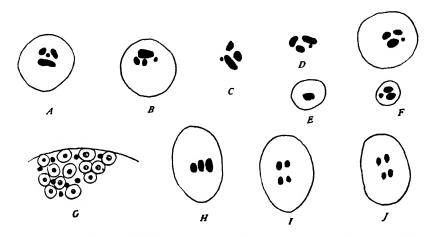


Fig. XXII Lachnus dentatus. A-D, F, second spermatocyte, equatorial plates; E, F (lower half), rudimentary cells; G, part of ovary showing rudimentary cells (black); H-J, second spermatocyte divisoin.

very small chromosome, besides two others in the reduced number. I was not without hope that this might prove an analogous case to that of Phylloxera caryæcaulis and that the smallest chromosome might be a small accessory in addition to the large one. This is, however, not the case as the following facts show.

Equatorial plates of the first spermatocyte division are drawn in Fig. XXI, B-I; side views of the dividing plate in Fig. XXI, L-P; and later stages in Fig. XXI, Q-X; Fig. XXII, F. In Fig. XXI, K-P, the division of the smallest chromosomes is clearly seen. The unequal division of the cell is shown in Fig. XXI, R-W.

Equatorial plates of the second division are shown in Fig. XXII, A-D. Here again the four chromosomes are seen. The lagging chromosome is now, proportionately to the other chromosomes, much larger. In two cases (Fig. XXII, E, F), the rudimentary spermatocytes are also shown, and a section (Fig. XXII, G,) through a follicle containing the second spermatocyte shows how conspicuous these rudimntary cells are at this time. In fact

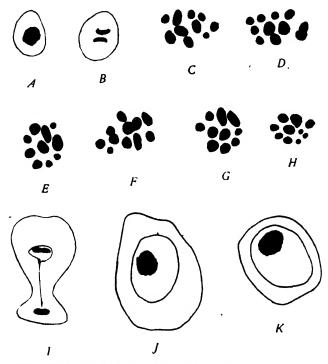


Fig. XXIII Chaetophorus viminalis! A, spermatogonial equatorial plate; B, division of same; C-H, equatorial plates of first spermatocytes; J, division of first spermatocyte; J. K, synapsis of chromosomes of egg.

they form one of the most conspicuous and easily seen features of such cysts.

Another species, Chaitophorus viminalis, produces sexual forms in the late summer. It is not favorable for study since the chromosomes are numerous, crowded, and one, or more, irregular

or possibly double. I count 9, 10 and 11, but the clearest cases (Fig. XXIII) give 10 chromosomes and this I believe to be the typical number. A late stage in the division of the first spermatocyte is figured in Fig. XXIII, I.

This species shows clearly, however, in the oögenesis a stage that resembles the synapsis stage of other insects. It is shown in Fig. XXIII,  $\mathcal{J}$ -K. These ova are found in the bottom of the ovary, and probably become the sexual eggs. If this were not the case, a synapsis stage would be passed through even in eggs that retain the full number of chromosomes. It has been stated in fact that this process occurs in the parthenogenetic eggs of aphids, but this requires confirmation.

# CYTOLOGICAL PARALLEL BETWEEN PHYLLOXERA CARYÆCAULIS AND SYROMASTES MARGINATUS

Wilson has pointed out recently the striking resemblances in the chromosomal cycle between these two species, and the comparison removes what might otherwise have proved to be a very anomalous condition in one of the two forms here described. The diagram which Professor Wilson has constructed (1909) will show at a glance the points of comparison.

The comparison depends primarily on the behavior of the two accessories and is independent of the interpretation as to their origin. Wilson adopts the view that in this case two chromosomes are the product of division of one accessory, rather than an original pair that now move to the same pole. In support of this interpretation he points to the series of species described by Payne in which one, two, three or four chromosomes form one group, with which a single chromosome still forms "a pair."

In Syromastes there are two accessory chromosomes of unequal size a+b as in P. caryæcaulis and these unite when they pass into the "female producing" sperm. In the female there are two pairs of homologous chromosomes, a+a, b+b. Presumably these pair with each other during synapsis and subsequently separate when the polar bodies are formed giving a+b for the egg and a+b for the polar body. The female sperm brings in

another a+b to produce the female; since male sperm lacks both a and b the male has only one a+b derived from the egg. This interpretation is exactly the same as that for P. caryæcaulis, except, of course, the male sperm being rudimentary only one kind of individual results from the fertilized eggs.

#### PART II

# CRITICAL REVIEW OF THE RECENT LITERATURE

## INTRODUCTION

Two contrasting views appear in an examination of the recent theories of sex determination, one qualitative, the other quantitative. On several former occasions (1903, 1905, 1906, 1907) I have urged the advantages of the latter interpretation as explanatory of the facts. By a quantitative interpretation, however, I do not mean that the female is simply male plus something else a view recently advanced by Castle, but that male and female are two alternative possibilities of the living material, which possibility is realized depending on quantitative factors. When these quantitative factors are internal there are produced in the germ cells those conditions that turn the scale one way or the other. The gametes are not, therefore, male and female, but contain certain factors which, when combined, give rise, in an epigenetic fashion, to one or the other alternative. By a qualitative interpretation I understand that there may exist in the gametes certain bodies or substances containing the materials either for a male or for a female. These bodies or substances are usually thought of as separated, "segregated" in different gametes at some period, and are then recombined in fertilization in such ways as to insure two kinds of individuals.

The qualitative and the quantitative hypotheses look at the problem of sex from different points of view.

In order to bring out the contrast of these alternatives I have attempted to give in the following pages an analysis of the results from both points of view without attempting to disguise my preference for the quantitative interpretation with important modifications of that view.

# THE CYTOLOGICAL EVIDENCE

The recent cytological discoveries may be said to have revolutionized our ideas concerning the possibilities of sex determination particularly within the group of insects, and more particularly within the hemiptera, to which group the phylloxerans belong. The discovery of the accessory by Henking in 1890, the further studies by Montgomery in 1898, by Paulmier in 1899, and by McClung in 1800 and 1900 laid the foundation for the remarkable discoveries made later. In 1902 McClung suggested that the two kinds of spermatozoa might be connected with sex determination and inasmuch as he observed that the number in the male was unequal he inferred that therefore the sperm containing the accessory produced the male while the sperm without the accessory produced a female. On this assumption the female group should contain one less chromosome than the male. The facts published in 1905 by Stevens and by Wilson showed, on the contrary, that the female contains one more, not one less chromosome than the male.11 Thus while McClung's general inference proved true, when the necessary facts were obtained, the further question raised by him as to the relation of sex to the accessory proved to be the reverse of his assumption.

The literature of the rôle of the accessory in spermatogenesis has been so often reviewed in recent years that there is no need to review it again.

In regard to the phylloxerans, two conclusions of importance are deducible from the evidence. In one species, P. caryæcaulis, there occurs an early union of two pairs of chromosomes and it is probable that these are the accessories. Second, the evidence that two whole chromosomes are eliminated in the male rests on the observation of the number of chromosomes in the polar body, as well as the absence of two chromosomes in the egg after the extrusion of the polar body, and lastly on the inference that two chromosomes must have been eliminated in the male line in order that the behavior of the accessories in the spermatogenesis can be made to conform with their behavior in the rest of the insects.

<sup>11</sup> When a pair of idiochromosomes is present the female contains the larger.

In regard to the last point, it should be noted that the only other alternative would be to assume that the accessories unite in the male to produce one, or two, chromosomes less than in the female. If this were true, however, there would be a heaping up of chromosomes in every generation unless all of the accessories were ejected from the sexual egg.

The discovery of Meves that in the spermatogenesis of the bee functional and rudimentary sperm are produced invites direct comparison with the somewhat similar facts in the phylloxerans. Meves has shown that at the first spermatocyte division the centrosomes move to opposite sides of the cell, a somewhat irregular spindle figure appears and the chromatin mass assumes the form of granules (chromosomes). There is no subsequent separation of the chromosomes into two plates. In fact, all present remain in the cell, which pinches off nevertheless at one pole a small nonnucleated mass of cytoplasm. At the second spermatocyte division the centrosomes move apart, the granular chromosomes appear again, and this time divide, but the cytoplasmic division that follows is very unequal. One of the resulting nucleated cells contains only a small amount of cytoplasm—the other large cell alone differentiates into a functional spermatozoon. Thus in the bee only one of the possible four spermatozoa found in other animals is produced. Mark and Copeland have also later obtained closely similar results for the honey bee. A somewhat similar series of changes takes place in the egg of Vespa germanica, according to Meves, and his main points have been confirmed by Mark and Copeland for Vespa maculata. Here also the first spermatocyte division is abortive, but at the second division there is an equal division of the cytoplasm (as well as of the nucleus) and two functional spermatozoa result.

Concerning the interpretation of these results Meves points out that, since the male bee develops from an unfertilized egg that has extruded its two polar bodies, it must contain the reduced number of chromosomes; hence one division of the spermatocytes suffices,

<sup>&</sup>lt;sup>13</sup> The fact that one rejects as not worth considering the view that chromosomes may be absorbed and disappear in this way shows how strong a hold has the idea of the continuity of the chromosome-material.

from Meves' point of view, to reduce the chromatin by one-half, and thus prevent its quantitative accumulation through fertilization.

In regard to the production in the bee of only one-fourth as many spermatozoa in proportion to its spermatogonia as other animals produce, Meves states that according to Leuckart the bee produces nevertheless from twenty-five to thirty millions of spermatozoa. If even half that number be assumed and if only a few (six to eight) be set free for each fertilization the contents of the seminal receptacle will suffice for three or four years at the rate of 150,000 to 200,000 eggs a year. "Eine doppelt so gosse Anzahl von Spermien wie sie resultieren wurde, wenn die zweite Reifungsteilung bei der Honigbiene ebenso wie bei der Wespe die Entstehung von zwei gleichgrossen Tochterzellen zur Folge hätte, wäre dem nach offenbar unnütz; es hat den Zwecken der Art besser entsprochen weniger Spermien zu bilden und diese besser aüszurusten."

The quotation shows that Meves looks upon the peculiar change in the bee as a sort of adaptation to produce fewer but better spermatozoa, but it must, I think, appear rather extraordinary that such a method of producing better results should have been evolved, especially, in the light of the fact that in all the animal kingdom, with the rarest exceptions here mentioned, no such betterment has taken place. On the contrary, I think one cannot but suspect that the results are connected with the peculiarities of reproduction and parthenogenesis in the bee, and when we find an analogous series of events in the phylloxerans associated in somewhat the same way with these peculiarities of reproduction, the suspicion grows almost to a conviction that the interpretation is to be sought in this connection.

Let us therefore look a little more critically into the case of the honey bee.

Starting with Meves' discovery that in the male bee the chromosomes are in the reduced number—the result not of a union or synapsis of chromosomes, but to the half number of single elements

<sup>&</sup>lt;sup>19</sup> Especially when the enormous number of eggs produced by the bee in comparison with the few in many other forms is taken into consideration.

being present—we can see how the first division should be abortive if we admit that at this division in many forms the separation of the paired chromosomes occurs. To Meves this interpretation would be meaningless since he does not accept the current view regarding the nature of the reduced chromosomes, but to those who think the evidence at hand points to such an interpretation of the reduction division, the assumption here admitted may make an appeal. The interpretation would mean that while the ordinary process of division is attempted it becomes abortive owing to the absence of the ordinary chromosomal behavior. Whether the result may not also have a real significance so far as the cytoplasm is concerned must be left an open question.

What meaning then would the second divison have? If the first had been, as assumed, the reduction division, the second would be an equational were all the chromosomes present. If, owing to the failure to separate the sex chromosomes in the first the process should take place in the second division, there might theoretically be produced two kinds of sperm, "male- and female-producing." If the former degenerates, all that become functional would produce females. Objections to this argument are evident. The fact that in Vespa both products of the second division become functional sperm is fatal to such a view—provided, as seems more than probable, the fertilized eggs of Vespa also all produce females.

The difficulties are not lessened by reversing the first assumption regarding the nature of the first spermatocyte division in the bee and wasp. In some groups of insects the first and in others the second division is supposed to be the reduction division. Now if we assume for the bee that the second is the reduction division we may appear to account for the production of a functional "female-producing sperm" (the "male-producing" being the rudimentary one); and if we assume for the wasp that the first division is the reducing one that separates male from female-producing cells—the latter alone dividing in the second—we might appear to account for the facts. In reality such an interpretation meets with more difficulties than it explains; it leaves unac-

<sup>&</sup>lt;sup>16</sup> There is no evidence from Meves' observations that an accessory passes out in the first spermatocyte division.

counted for the failure of the division of the chromosomes in the first division of the bee, and, worse still, it fails completely to show how in the wasp the first division is a reducing division since all the chromosomes are retained. Whatever evidence the future has in store for us concerning the second division of the spermatocytes, the failure of the first division seems in all probability to be connected with the presence in the male of the reduced number of chromosomes.

Schleip finds for the ant, Formica sanguinea, that two polar bodies are given off from the egg as Henking had previously determined for another species, Lasius. The reduced number, 24, of chromosomes, appears in all eggs. Two polar bodies are formed, and into each 24 chromosomes are ejected. The female pronucleus contains still 24 chromosomes. After fertilization about 48 chromosomes can be counted, and the inference is plain that the reduced number, 24, is brought in by the sperm. These facts fit in with the results for the bee and go to show, taken in connection with the recent results on spermatogenesis, that the male contains the reduced number of chromosomes, the female the double number.

Lams has also found in another ant, Campodinotus herculaneus, that the first spermatocyte division is abortive and the second normal. No evidence of a lagging chromosome is to be found in the admirable side view of spindles given by Lams.

It has been generally taken for granted that in the ant as in the bee unfertilized eggs produce only males and this view has the support of observations by Forel, Lubbock and Field. On the other hand Wheeler has recently drawn attention to other observations showing conclusively that the unfertilized egg in certain species may produce females. Tanner obtained workers of Atta cephotes from an artificial nest composed of workers. The same nest produced numerous males also. Reichenbach obtained large numbers of workers of Lasius niger from the eggs of workers laid in an artificial nest. Males, too, appeared synchronously with their appearance in nature. Mrs. Comstock has also obtained

<sup>&</sup>lt;sup>16</sup> Wheeler, W. M. The Origin of Female and Worker Ants from the Eggs of Parthenogenetic Workers, Science, xviii, December, 1903.

workers from workers' eggs in the American variety of Lasius niger.

These observations make not improbable the view that the unfertilized eggs in certain species of ants can produce females, but unfortunately we do not know whether two polar bodies are extruded in such cases. Whether this happens or not, the facts indicate that before the extrusion of polar bodies the eggs have the dual potency of producing males or females. Such a conclusion makes it probable that the bee's egg too may have the same possibilities, only here the results are more one-sided, since after extrusion of the polar bodies the resulting egg is male determined. It would therefore be erroneous to conclude that the eggs of the bee are male before the polar bodies are extruded.

This case of the honey bee has been one of the greatest stumbling-blocks that modern theories of sex have met. One course in avoiding the contradictions here found has been to deny the facts, yet the facts seem to me to be as clearly determined as any other in this field, and while none of them may be final, yet it is unfortunate to reject one body of evidence because it does not fit in with conclusions derived from the other.

The unfertilized eggs all develop into males. It is assumed that all the eggs are therefore male producing, and that the sperm brings in the female determinant. But there is another theoretical possibility. The sex of the egg may not be determined at first. If it is not fertilized, it becomes a male; if fertilized, a female. The absence of fertilization or the act of fertilization may be the determining factor; or if the result is attributed to something lost or retained by the egg, such a result might be referred to the polar body formation which responds to the presence or absence of the sperm. Such are the difficulties met with on the assumption that the sex determinants exist in the egg or sperm or in both. None of these are necessary if we account for the results on the simple assumption that one nucleus means male, two mean female, which is the view that I adopted in 1903.

In another hymenopteron, Nematus ribesii, the facts described by Doncaster are so anomalous that at present it is quite impossible to bring them into harmony with what takes place in the bee, wasp and ant. It is true that two polar bodies are given off from all eggs as in the bee, according to Doncaster, but he describes two types of eggs, those in which reduction occurs and those in which it does not occur. His suggestion that only those eggs are capable of fertilization in which reduction occurs may appear to account for the sexual eggs, but it still leaves unexplained how two polar bodies can be extruded from the egg that produces a male without a reduction of the chromosomes.

In an earlier paper Doncaster called attention to certain facts, that appear to be well established, showing that in the saw-flies (Tenthredinidæ) virgin eggs produce males in some species, females in In Croesus varus "the male is not certainly known, one observer only having described it, and all agree that females alone arise from virgin eggs." It should be recalled in this connection that Doncaster finds two polar bodies given off from this egg. is driven to assume, in consequence, that both divisions are equational, but this is obviously an argument ad hoc and out of harmony with the general trend of modern cytology. His observation, that two of the three polar bodies fuse, might be brought into harmony with the similar observation made by Petrunkewitch, and if, as the latter believes, the fused product makes the reproductive organ, the germ cells would then have the double number of chromosomes and reduction be possible in the egg. But there is serious doubt in regard to Petrunkewitsch's conclusion and Silvestri's observations on certain Chalcidæ show, in fact, that the polar bodies take no part in the development of the reproductive organs of these hymenoptera; yet the facts of development are identical with those in the bee. There is obviously something that is still lacking in these observations on the saw-flies, that makes it impossible to harmonize the results with the facts in other forms.

Doncaster points out that in Pœcilosoma luteolum the male is extremely rare, "and Miss Chawber of Lyndhurst tells me that she has bred thousands of this species for several years in succession without obtaining a male and without finding any diminution in the fertility of the females."

Hemishroa rufa produces "chiefly females from virgin eggs but occasionally males are produced."

According to Cameron, Nematus pavidus gives rise only to males from virgin eggs, and Miss Chawber<sup>16</sup> has reared males only from virgin eggs of Nematus lacteus.

Von Siebold found only 13 females among 1700 males from the virgin eggs of Nematus ribesii. Doncaster finds that many virgin eggs do not develop, the percentage being lower than that for fertilized eggs.

The fact that virgin eggs in several species of saw-flies produce males and females, or either males or females proves conclusively that the egg of these hymenoptera may produce both or either sex. This result seems to me to indicate with some probability that in general the eggs in this group have the double possibility and that some mechanism exists by means of which the virgin egg is thrown, as it were, in one or the other direction towards the female side in Pœcilosoma luteolum, towards the male side in other members of the group and in the bee. What turns the balance in these cases is still unknown, but it is evident that a closer scrutiny of the polar body formation and the events that immediately follow is needed. In the "gall-flies" (Cynpidæ) there are several species known in which two generations alternate. One generation is composed exclusively of females that lay parthenogenetic eggs from which males and females hatch. Here once more we find eggs which without being fertilized produce both sexes. In the generation composed of males and females the fertilized eggs-presumably all are fertilized-produce only females that are parthenogenetic as we have just seen.

# THE INFLUENCE OF THE ENVIRONMENT ON PARTHENOGENETIC REPRODUCTION

It is in connection with parthenogenesis that the results here recorded for phylloxera and aphids have particular interest, for it is in the parthenogenetic egg that sex is determined. In two other groups of animals the relation between sex and parthenogenesis is so similar to that in phylloxerans and aphids that comparisons must inevitably be made.

<sup>16</sup> See Doncaster, footnote, p. 563.

In the phyllopods, Daphnia and its related genera, that we may for the sake of brevity refer to as daphnians—it has long been known that the fertilized eggs produce only females, while, from parthenogenetic eggs males or sexual females are produced. Weismann's classical and important observations on this group led him to the general conclusion that the life-cycle-its extent and phases—is the result of an internal mechanism that has become adapted, so to speak, to those conditions that each species is most likely to meet with. On the other hand the observations of Issakowitsch go to show that external conditions affect materially the phases of the life-cycle in the species Simocephalus vetulus. On Weismann's view the parallel between the daphnians and the phylloxerans is obvious, but in how far they are the outcome of similar internal factors we do not know. On Issakowitsch's view the internal mechanism is largely affected by the environment, but whether the effect is "sex-determining" or only determines when the parthenogenetic cycle gives place to the sexual phases remains to be examined. In daphnians one and the same individual may produce eggs that become parthenogenetic individuals, or eggs that become covered with a thick coat and require fertilization in order to develop, i.e., sexual female eggs; or eggs that become males. In the first case, the mother produces by means of parthenogenesis, parthenogenetic young; i.e., she may be said to be a parthenogenetic individual; in the second place, the same mother produces a sexual egg, she may therefore be said at the time to function as a sexual female; in the third place, she produces parthenogenetically a male, she may at this time be looked upon as a parthenogenetic female that produces males—she herself, of course, not being a male, but a male-producer. The point that I wish to make in this connection is this; that whatever the mother brings forth, she cannot be said to change her sex, nor if the conditions determine that she ceases to bring forth parthenogenetic individuals and produce sexual eggs or males can those conditions be strictly said to be sex-determining provided they lead equally to the production of males and sexual eggs. The change is from parthenogenesis to sexual reproduction and the factors might be said to be sex-producing, not sex-determining; since by

common usage sex-determining means to call forth a male or a female.

Issakowitsch found that by keeping the daphnian (Simocephalus vetulus) at a temperature of 24° C. he obtained during six generations a succession of parthenogenetic forms, about 500 individuals in all. At the end of three and one-half months the culture died out. In another culture carried through six generations composed of 85 broods of about 500 animals in all, the great percentage of individuals were parthenogenetic, but here and there males and winter eggs appeared. The longer the culture was kept at 24° C. the stronger was the tendency to produce only parthenogenetic females. An examination of Issakowitsch's table brings out a point not mentioned by him, namely, that along certain lines of descent there appears to be a stronger tendency to produce males than along other lines.

At room temperature the results are strikingly different, for even in the first generation males and winter eggs appear so frequently that the cultures disappeared in one or two generations.

In the cold the cultures were shorter owing to a stronger tendency to produce males and winter eggs.

The results seem to show that sex-production is determined in these animals by temperature. Food was equally supplied in all cultures yet Issakowitsch thinks that in the final analysis food conditions bring about the result in connection with temperature which only acts indirectly. In order to examine this question he kept two sets of animals under the same temperature conditions viz: 24° C. One was starved, the other fed. The former produced males and sexual females at once. Issakowitsch concludes that food rather than temperature per se is the sex-determining (sexproducing) factor. He believes that the following facts strengthen his view. If the winter egg is not fertilized its contents are absorbed, the empty shell being thrown off. Issakowitsch thinks that this winter egg supplies the ovary with nourishment. In consequence the next brood is parthenogenetic. After this the conditions between temperature and food are again established, and a sexual brood results at a low temperature.

A correlation is found to exist between the kind of egg pro-

duced and the condition of the oviduct. In the young female the lining cells are all filled with fluid which is lost when the parthenogenetic eggs enter the tube—presumably supplying them with nourishment. After the eggs are set free in the brood chamber the lining cells again enlarge, etc. But if a winter egg is forming the cells remain flat and inactive. The winter egg grows at the expense of the surrounding cells in the ovary. Issakowitsch's interpretation is that when the nourishment is good the oviductal the cells enlarge; when poor, the egg must get its nourishment from immature cells of its ovary and the lining cells remain undeveloped.

His general conclusion is "that since at a low temperature the assimilatory activity of the cells is lessened, and since the activity of a growing egg is more intense than that of any other cell, we must conclude that at a low temperature the conditions are unfavorable for nourishment of the egg in the ovary. We expect therefore to develop a winter egg or a small male egg."

However doubtful it may appear that Issakowitsch has given a complete account of the methods by which the results are affected, his experiments seem to show that external conditions are instrumental in affecting the transition from one mode of reproduction to another. There is nothing in his results that throws any light as to how in one case a male is produced, and in the other a sexual egg. In fact we do not know as yet whether all the ovarian eggs are alike or whether two (or three) sorts of eggs exist corresponding to the two or three kinds of individuals that are produced. there are such eggs, external conditions might determine which egg develops. Or if there are only two kinds, sexual (male and female) and parthenogenetic, the conditions might determine which one at a given moment developed ("accident" alone determining whether if a sexual germ is set free, it be male or female). On the other hand since in the end all three types would be traced to the parthenogenetic egg that gave rise to the mother, no fundamental distinction can exist for them, since we should still have to explain how from the parthenogenetic egg the three hypothetical types arose during development.

There are certain facts in connection with the life-cycle of the aphids that appear to be similar to those here described for daph-

nians. When the sexual forms appear the leaves have in many cases begun to dry up, or at least to harden so that nourishment may be more difficult to obtain. In the rose aphid the sexual forms are found on the lower, older leaves, while the parthenogenetic individuals are on the terminal young buds and younger leaves. If these are brought into the green-house they continue to produce parthenogenetically; the leaves of plants grown under such conditions remain soft, many young leaves are present and the terminal buds continue to grow. It seems very probable that temperature has no direct influence on the life-cycle in the aphids, and the facts here given suggest that the change may be connected with conditions of nutrition.

The life-cycle of the rotifer, Hydatina senta, is similar in many ways to that of the aphids and phylloxerans. Here, too, all fertilized eggs become females, and these females produce by parthenogenesis a succession of parthenogenetic individuals. Some of these may produce males, others sexual eggs, but the same female never produces more than one kind of egg. Maupas's work on Hydatina senta seemed to show that a high temperature increases enormously the proportion of male-laying females (97 per cent) not by changing the character of the females so that a different kind of egg is produced, but by affecting the character of the laid egg, or its method of development, so that it becomes a male-laying individual. Conversely Maupas believed that a low temperature greatly increases the output of female-laying individuals (95 per cent). Nussbaum, Punnett and Whitney, who have repeated the experiment, find no evidence that supports Maupas's view. Whitney points out how Maupas's facts may be correct, but his conclusions wrong. At a high temperature the male-laying females are not so much affected as to their output of eggs as are the femalelaying individuals, hence the percentage of the male eggs will be proportionately increased. Furthermore while the average proportion of male-laying females is about 22 per cent some individuals produce fifty per cent of these individuals, others only two to five per cent. That these individuals are not fixed in respect to sexproduction was also shown, for a low male-producing strain may later become a high one. It is difficult to understand how such

variations are brought about unless in some way the conditions under which the animals live produce an influence on the results. It should be repeated here again that if such an influence exists its effects may be to cause a change from a parthenogenetic to a sexual method of reproduction, i. e., it would be sex-producing, not sex-determining. In Hydatina, in fact, "the cause" of sex-determination is fortunately fairly well made out. If a young individual destined to lay male eggs is fertilized, she lays henceforth winter eggs and nothing but winter eggs. clear that the presence of the spermatozoon within the developing egg determines its fate. The conclusion is rendered more probable from the fact that Lenssen observed in Asplanchna that male young and winter eggs occur in the same individual. Whitney's observations show that the spermatozoon that has entered an egg is present throughout part of the growth and all of the maturation period. It may produce its effect either during growth, or maturation, or its effects may be due to the presence of a double number of chromosomes in the embryo causing it to develop into a female. There is a point here of capital importance that has not as far as I know been noticed, viz: that the influence of the sperm must occur while the germinal vesicle is still intact. This is shown by the size relations of the egg; the male egg is smaller than the winter egg, hence the latter must enlarge further if it is to become a sexual egg. The presence in it of an additional nucleus might on first thought be assigned as the cause of its special enlargement, but when we recall the fact that the sperm nucleus remains in a condensed condition up to the maturation period, this mode of explaining the result will not appear in so favorable a light. It seems probable that in Hydatina the presence of the sperm in the egg acts in some unknown way so that a sexual egg results; and that the result need not be connected with the functional activity of the sperm in the sense that it assists the processes of assimilation in the egg. The fact, however, that the egg becomes larger is of theoretical importance, since this probably means that at the same time (but not in consequence of the enlargement) it is determined as a sexual egg. Even if some corresponding regulation should occur later in the chromosomes to complete the chain of events, the important conclusion would remain, that the preliminaries of sexdetermination—and possibly therefore its cause in the sense of inaugurating a sequence of events—takes place in the egg of Hydatina before the maturation of the eggs. These preliminaries involve moreover the question of sex-determination and not simply sex-production.

Lenssen's observations on the maturation of the egg do not seem to be entirely correct, although his general conclusions that the same number of chromosomes are present in the male egg and in sexual eggs, and that double that number are found in the parthenogenetic eggs, is sound. The recent results of Whitney place this conclusion on a surer basis. Whitney points out that the parthenogenetic egg extrudes one polar body, the male egg two; that the sizes of the individual chromosomes in the male egg and in the sexual egg are twice that of the chromosomes in the parthenogenetic egg. These facts leave little room to doubt that a reduction in the number of the chromosomes of the male egg takes place preparatory to the formation of a male. Hence the possibility of fertilization of such an egg becomes intelligible, since the reduction is a preparatory step to fertilization in the sexual egg. The fact too that the reduction occurs in the male egg (that develops parthenogenetically) shows that the entrance of the sperm in the sexual egg has nothing whatever to do with the determination of reduction in that egg, and this substantiates the conclusion reached above that the preliminaries for sex determination go on irrespective of the presence of the sperm. In this instance, however, the process only involves reduction that is common to both male and sexual female eggs.

Another fact emerges from Whitney's observations—the male egg, as well as the parthenogenetic egg, is produced in the presence of all of the chromosomes—as in the phylloxerans. So, too, is the sexual egg, plus, however, the chromosomes of the male—which proves too much for the chromosome view, unless the compact sperm be assumed to have already become functional. But this assumption cannot be made for the phylloxerans.

If, after reduction and the extrusion of two polar bodies, in the male rotifer, we suppose that the male has half the total number of chromosomes, the case is strictly comparable to that of the bee. Theoretically there should not be two spermatocyte divisions but only one true division. It remains to be seen whether this prediction prove true.

The male rotifer develops in the presence of half the full number of chromosomes; in the phylloxeran the male develops with the full number minus the accessories. The sexual eggs in both forms and the parthenogenetic eggs contain alike the full number during development, but preparatory to development the sexual egg reduces its number of chromosomes which is made good by the addition from the male.

For the bee it has long been disputed that all the unfertilized eggs are male producing (fertilization turning them femalewards). Certain authors maintain that there must be two kinds of eggs and that only the male eggs can not be fertilized. The case of Hydatina shows that an egg destined to produce a male may, if fertilized, become a female, which is a case analogous to that of the bee except that the determination by fertilization seems to come earlier in Hydatina.

The third type in which large female-producing eggs exist and small male-producing ones, is that of Dinophilus apatris, as Korschelt has made known. In one important respect the form differs from the other two, viz: both eggs are fertilized (presumptively) and both give off two polar bodies. This shows that the result is independent of the sperm unless selective fertilization occurs. If selective fertilization does not occur, it is evident that sex is determined in the egg when all the chromosomes are present, for the two sizes of eggs exist when the eggs are laid. Whether subsequently changes take place that reduce the amount of chromatin in the small egg we do not know. A series of experiments were carried out by von Malsen that show that more female eggs are laid at a low temperature than at a high temperature. In the cold the proportion is about I male to 3.5 females; in the warmth, about I male to 1.7 females. These results are interpreted by von Malsen to mean that sex is determined by external conditions. The effects are produced, he believes, not directly by the temperature but by the influence of nutrition—the relation between the

metabolism and the temperature determining the result. In support of this conclusion he cites some experiments in which the animals were starved when the percentage of males was increased. This conclusion is open to one serious criticism. If the condition of nourishment determines the number of large eggs that can develop, the proportion of the two kinds of eggs that mature if two kinds really exist at an earlier stage—will be altered. would mean not that sex is determined by the nutrition of the parent but only that a well-nourished parent can produce more female eggs than one that is starved. This is precisely the state of affairs shown by the phylloxerans. Poorly nourished females produce only one egg, but this is of normal size, while well-nourished females may produce eight to ten or more. Such differences would seriously affect the sex ratio if the males are somewhat less affected, owing to the small size of the egg. We have no means of knowing at present whether the large egg of Dinophilus apatris formed by the fusion of several ovarial cells is large on account of the number of cells that unite to produce it, or whether, being already a female egg, it absorbs a larger number of cells than does the male. One fact speaks strongly in favor of the view that the result is not due simply to the number of cells absorbed but to the determination of the egg before or at the time of absorption, namely, the great difference in size of male and female eggs. If the result were due to the factor of absorption alone, we should expect to find a graded series, not a dimorphic condition. evidence, while as yet not decisive on this point, indicates, nevertheless, that there must be already in the ovary two kinds of eggs prior to the absorption period, and that the nature of the egg determines how much of the surrounding material it will absorb. this view prove correct, sex in Dinophilus apatris must be determined in the egg long before the maturation stage is reached. The conclusion is in harmony with the facts here described for Phylloxerans.

# THE PROBLEM OF THE THREE CHROMOSOMES

In those insects where the spermatogonium of the male has one accessory, the oögonium of the female has two chromosomes, homologous with the accessory of the male. These three chromosomes form pairs whenever they meet (in the female only). To all external appearances they are identical. We have no evidence to show that they are functionally different, but the qualitative interpretation demands that they must be different if sex is determined by them; if not sex is a purely quantitative result. If one of these three is female determining and the other two male determining, the results, so far as sex goes, can be explained only on the theory of selective fertilization. This hypothesis rests, therefore, on two assumptions, neither as yet verifiable.

On the assumption, that the three chromosomes consist of two male chromosomes and one female (despite their external identity) we can work out the problem with the help of selective fertilization as follows for those cases where an accessory chromosome is present in the male.

We may represent the male determining chromosome by M, the female determining chromosome by F. Hence,

Sperms
$$\frac{M}{O} \longrightarrow F = MF \text{ (female)} + MO \text{ (male)}$$

For the case where there is a fourth smaller chromosome (f), this appears only in the male line, and the formulæ would be

Sperm
$$\frac{M}{f} \xrightarrow{Eggs} MF \text{ (female)} + Mf \text{ (male)}$$

In the majority of cases where the fourth chromosome is equal in size to the other three, the formulæ are

Sperm
$$\frac{M}{F'} = MF \text{ (female)} + MF' \text{ (male)}$$

Unless F' is different from F both results would be the same and only one sex, the female, would arise; but if F', while the potential

bearer of the female character, is weaker than F, or in modern terminology, the dominant F has become a recessive F' (with respect to M), the results can be accounted for. Expressed in phylogenetic terms, originally only one kind of individual developed as a result of union of F and M. Gradually two kinds of individuals arose owing to one of the F determinants becoming weaker than its opponent, M. In time this F became smaller or vanished, because it lost all real function except as the bearer of latent female characters in the male. In other words whenever four sex chromosomes exist the male carries potentially (recessive) the female characters, wherever the fourth chromosome has dis appeared, as in certain insects and spiders, the male is a homozygous male. This scheme is advanced merely in order to show how, on the chromosome theory, the graded series shown by the fourth chromosome might be consistently worked out. The view assumes both reciprocal fertilization and sex primordia that "segregate" in the gametes. It ignores the fact that no differences between F' and F have been seen. It assumes that sex primordia exist both in males and females and that there are two classes of males in different species, some pure males, others mixed. It encounters the objections that may be raised against the view that the egg determines in all cases the sex, because it contains the dominant male and female primordia.

The problem is simplified if we assume that the three chromosomes are identical, in which case the result is quantitative. Femaleness is only twice maleness in the sense that when one of the three bodies is present a male develops, when two a female. It may be urged in opposition to this conclusion (1) that the female may develop male characters; (2) that the difference between the male and the female characters may depend on qualitative differences as shown by injecting sperm extracts into the female that call forth male characters; (3) that in mosses the male and the female may develop with half of the chromosomes present; (4) that in the phylloxerans the producers of male eggs and female eggs appear with the full number of chromosomes present; (5) that the function of the mate of the accessory when it is present is ignored. These objections may possibly be met by the following considerations:

- I The female develops male characters (by which is meant secondary sexual characters not spermatozoa) in old age or after castration. This may mean that these secondary characters owe their suppression in the female to materials manufactured in the ovaries. In insects, however, it has been found that the secondary characters of the male do not appear in the female after castration. Gynandromorphism, that occurs in this group, may depend on a different relation and will be considered later.
- 2 The primary qualitative differences between the sexes have never been changed by injection, They represent the fundamental alternatives of the protoplasm and quantitative factors only determine which alternative is realized. The secondary characters of the male that arise in the female by injecting male extracts can be explained if the injected substance overcomes the inhibiting effects produced by the products of the ovary.
- 3 In mosses only one kind of sporogonium exists, hence as pointed out, the case is not parallel. Waiving this distinction, the problem in the mosses is concerned with the development of two kinds of gametes which may depend on some unknown quantitative relation existing in the reduced groups of chromosomes—if chromosomes here represent the material factor of sex determination.
- 4 In the phylloxerans all the chromosomes are present when the male and the female egg develop, yet a change in their grouping has been demonstrated in one case at least. Whether this in itself will account for the result is not evident, but the change is concerned with the production of two kinds of eggs not necessarily with the production of male and female. The male develops only after two chromosomes are extruded.
- 5 When the mate of the accessory is the same size as the accessory—in other words when no accessory exists—we have no obvious difference in the chromosomes by which to explain sex. Yet differences may exist that have not yet been found, as Boveri has shown, in the case of the sea urchin. Until such facts become known, however, this is a real objection to the quantitative interpretation, and unless such facts are discovered it is fatal. The absence of data on this point is not due to lack of observations,

for, in several insects where the number of chromosomes is small the pairs have been closely studied by Wilson and no differences discovered.

In 1906, on the basis of his own observations relating to the number of chromosomes in the male and female and of the similar results of Stevens, Wilson discussed very fully two alternative views of sex determination. The first rested on the assumption of male-determining and female-determining chromosomes. He pointed out that this hypothesis involves the idea of selective fertilization, in favor of which Cuénot's hypothesis for yellow mice is cited. As a second, "alternative hypothesis" Wilson suggested that the "differential chromosomes may perform a definite and special function in sex production without being themselves specifically male-determining and female-determining, or even qualitatively different save in the degree of their special activity (whatever be its nature). The kind of activity that produces a male will, if reënforced or intensified, produce a female."

In the case of the bee he points out the difference may depend on fertilization or its absence. Where an unpaired accessory exists, as in Anasa tristis, the presence of this one, and of another of the same kind (in the egg) produces a female, while in the male one of these alone is the cause of that sex developing. But when, as in other cases, there is no unpaired accessory, but a corresponding pair of equal sizes, the quantitative result no longer holds. Therefore Wilson suggests that one member of the pair in the male may show less activity than its mate. In consequence the egg fertilized by such a sperm becomes a male, and the weakened chromosome is confined to the male line. This suggestion is somewhat similar to the one discussed above in which the weaker chromosome is represented as the female determiner (F'). In a later paper, to be referred to in another section, Wilson suggests a third view differing from both of these, but more nearly akin to the second one here described.

### THE BOTANICAL EXPERIMENTAL EVIDENCE

The important experiments of Blakeslee, of the Marchals and of Correns on plants have an important bearing on the problem of

sex in animals, since many points of resemblance appear in the two great divisions of the living world.

Blakeslee recognizes two groups of moulds; viz: homothallic in which the thalli are all equivalent, conjugation taking place between parts of the same thallus or between any two thalli; and heterothallic in which the thalli are of two sorts, designated by the symbols (+) and (-). If spores are taken from the (+) or (-)strain they produce a strain exactly like the parent. Phycomyces has been cultivated in this way through 107 generations, and Mucor mucedo through 106 generations without change in their sexual behavior. It appears that the (+) and the (-) strains perpetuate themselves indefinitely as such. If a (+) and a (-) strain are brought together zygospores are produced by the union of equivalent parts from each thallus. If two (-) strains meet, or two (+) strains no sexual process occurs. From the zygospore a sporangium is formed containing many spores. It is here that the discoveries are extremely important, for the spores in the same sporangium are some male, others female.

Another mould Sporodinia is homothallic, the mycelium (gametophyte), the germ tube (sporophyte), and the sporangia are all alike. In other words from the zygospore a sporangium arises containing only one kind of spore which produces a homothallic mycelium. There is no visible separation here into (+) and (-) elements, even although the conjugation phase of the mycelium be so interpreted. Such a form suggests the conditions found in an hermaphroditic animal, although the two kinds of germ cells in the latter present an external difference.

A third type, Mucor mucedo, has separate (+) and (-) mycelia. By their union a zygospore results which gives rise to a sporangium containing in some cases only (-) spores (to judge from their mycelial product) and in other sporangia only (+) spores. Both sorts of spores do not appear in the same sporangium despite the fact that the zygospore has been produced by the union of a (+) with a (-) strain. This remarkable discovery presents a quite different phase of the sex question. The case is paralleled, as Blakeslee points out, among the flowering plants by such a form as the Lombardy poplar, where the fertilized egg produces in some cases a

male tree, i. e., one producing only pollen, in other cases a female tree, i. e., one producing only ovaries. On this view any pollen grain could fertilize any egg, for all the eggs are of one sign and all the sperm of the opposite.

What determines whether the fertilized product gives rise in one case to a female and in another to a male is quite unknown. The three types of sexual reproduction represented by these three moulds, Phycomycetes, Sporodinia and Mucor, illustrate three important phases of sexual reproduction; the first shows that male and female strains develop in the same sporangium; the second shows that no dissociation occurs in the sporangium; and the third that one sporangium contains spores only of one sign and another of the other sign. Either the first or the third types might be made the basis for comparison with unisexual animals; if the first, there would be (-) and (+) sperm, (-) and (+) eggs, with reciprocal fertilization; if the third, all the sperm would be of one sign, all the eggs of the other sign; any sperm could fertilize any egg. Unfortunately we lack evidence for animals that show to which type they belong. Both alternatives will be considered later. be noted that the (+) and (-) signs are used with respect to fertilization, not with respect to the kinds of "sporophyte" that arise from the zygospore. Externally these sporophytes in moulds are alike whatever their physiological possibilities. Sex is used in animals and plants with regard to the kind of sporophyte that results from fertilization; these are of two kinds, male or female. If the third type of reproduction represents the transition to the higher type, two facts of much importance will be noticed; first that the male gametes all have one conjugation sign, and the female gametes the Hence every sperm can fertilize every egg. Sex therefore relates to the character of the sporophyte, rather than to the sign of the gametes; yet one sign is associated with one sex and the other with the opposite. The second fact is this; since opposite signs must unite the product is hybrid (+ and -) yet only one sign comes to development in the male, and the other in the female. Whatever determines the sex, therefore, must also determine the sign of its gametes. If the former is a quantitative relation, the latter must be also.

In the dioecious mosses the important experiments of Elie and Emile Marchal show interesting sex relations. In Ceratodon purpureus, Barbula unguiculata and Bryum argenteum each protonema gives rise to male or to female "flowers," i. e., the sexes are separate. When the oöspore is fertilized by an antherozoöid the resulting sporophyte produces spores which produce again the male or the female protonema. The Marchals found by sowing spores from single capsules that some of the spores produce male protonema, others female. In current phraseology this means that the fertilized oöspore contains both sexes combined and these are "separated" again, in the spores or, put in another way, the factors that produce the male and the female sex cells are both present in the oöspore generation and are separated when the spores are formd.

The experiments prove that the spores are not hermaphroditic, i. e., capable of giving rise to male and female buds on the same protonema; they leave open the question whether the sex is predetermined in the spore or whether the protonema gradually develops one or the other sex during its life in response to external conditions.

This possibility was tested by cutting off pieces from a protonema of known sex and raising them under very diverse conditions. Such pieces regenerate a new protonema. These secondary protonemata are always of the same sex as that of the original protonema. The evidence is still not final for it might be claimed that sex once determined at a critical stage by external conditions is irreversible in later stages, until the critical stage is again reached.

In a second paper the Marchals give some further experiments. It has long been known that the tissues of the sporophyte will produce a protonema if separated from the parent plant, and put under favorable conditions. Since these tissues result directly from the fertilized egg, they contain the full number of chromosomes, and, theoretically at least, also the double sex potentiality. The results show in fact that the same protonema produces both male and female flowers. The details are worth careful scrutiny. The first formed flowers were male in large excess, 1: 25.8. Later 1: 8.7 and finally 1: 7.8. This predominance of male elements

was very marked. It was equally well seen in the rare hermaphroditic flowers that contained both antheridia and anthegonia. In a very small number of cases the flowers were exclusively female.

Experiments were tried with the sporophytes of purely male flowers, mixed flowers and purely female flowers. Protonemata were obtained from each and the sex of their flowers recorded. In all cases the result was the same as in the first generation, irrespective of the kind of sporophyte utilized. Flowers of the three kinds appeared in all, the males in excess, even when a purely female flower had been used. Evidently the factors that determine male and female flowers are not decisive for the kind of gametes they produce. The results show clearly that the tissues of the sporophyte carry the conditions for both sexes, and that these become "separated" when the spores are formed.

It is perhaps not without significance to observe that the male flowers appear some time before the female ones, although the protonema is bisexual as subsequent results show. If this early suppression should continue throughout life the result would be difficult to distinguish from a male plant.

The Marchals point out that since the diploid or double number of chromosomes is present in all the cells of the sporophyte and the reduction occurs when the spores are formed, the "separation" of the sexes must occur also at this time. When we recall that in animals the "separation" of the sexes also occurs according to some current theories at the reduction period it becomes a matter of extraordinary interest to determine the meaning of this phenomenon. No less striking is the fact that with half the number of chromosomes some of the spores are male producing, others female producing. The product of the sexual union of the germ cells derived from these spores is a hermaphrodite. If we are justified in extending to animals the general point of view here reached, we might conclude that at the reduction period in the egg as well as in the sperm a "separation" of the male and the female factors takes place. If we assume that it is a matter of "chance" whether the egg or its polar body become a male or a female, then selective fertilization follows, but even with this assumption it would still remain a puzzle what determines the sex of the resulting hybrid.

We might conclude on the other hand that the egg always "retains" one sex factor and the polar body the other (while there are two kinds of functional sperm); the egg would then be homozygous but if two kinds of sperm are produced one must be functionless unless like can fertilize like. The third possibility shown by the moulds does not seem to appear in these dioecious mosses; viz: where one sporophyte produces only male spores and another female spores. Moreover in mosses all sporophytes are alike, while in animals and in some dioecious plants two types of sporophytes exist, males and females. If in these all the sperm are males (+) and all the eggs female (-) direct comparison with the mosses cannot be made. For the sake of clearness it may be well to keep these contrasts in mind.

The chromosomal cycle in ferns shows apparently that there is no necessary connection between the reduced number of chromosomes and the segregation of sex.18 Each spore produces a prothallium-homologous with the protonema of mosses-with the reduced number of chromosomes in all of the cells. Such a prothallium may produce both archegonia and antheridia, the former containing each an ovum, the latter spermatozoöids. This fact shows that the gametes of one or the other sex are produced long after the reduction has occurred, and on one and the same plant. If it can be shown that the spermatozooids of a prothallium can fertilize the egg-cell of the same prothallium, then they must be of The fact that in certain ferns it has been possible opposite signs. to suppress the development of the archegonia or of the antheridia by changing the external conditions—a result that Hertwig and Whitney have also obtained in Hydra—obviously 'does not show that the sex has been changed, but that in a hermaphrodite form certain conditions are more favorable for the production of one or the other gamete.

Previous to the recent agitation concerning the sex of the gametes and their reciprocal fertilization, it was generally taken for granted by zoölogists and botanists that any sperm may fertilize any egg.

<sup>&</sup>lt;sup>18</sup> In hermaphroditic prothallia the reduced group of chromosomes might be supposed to contain both elements that are separated only at the time of formation of the reproductive organs or of their germ cells.

In other words, the sex of the gametes as applied to conjugation has been merged into the sex problem of the sporophyte. At the present time, in fact, several recent theories of sex, as in Castle's latest view, do not hesitate to make a male gamete (sperm) fertilize a male gamete (egg). We meet here with an apparent contradiction in terms by using the same words, male and female, for the higher and the lower forms. The results can be harmonized if we admit that by sex in higher animals and plants we refer to a different condition from the sign of the gametes concerned in the act of fertilization. All sperm must then be of one sign (+ or -), all eggs of the opposite sign (unless reciprocal fertilization be assumed). But on the view that the female (or the male) is heterozygous in regard to its gametes, i. e., a + (or a - ) gamete may carry either a male or female sporophore-determinant, what then determines the sign of the gametes? This question is left unanswered or ignored. Evidently the result cannot depend on its association with the male or the female since no such relation is recognized on this view.

#### MENDELIAN TREATMENT OF THE SEX PROBLEM

The presence in animals and plants of two kinds of individuals, males and females, has led to several attempts to explain their presence according to Mendel's Law. Sex is treated as a unit character not different from other unit characters in its heredity. Correns' experiments give the best basis for an explanation along these lines. The more speculative writings of others will be considered later.

Bryonia alba is monœcious; Bryonia dioica is diœcious.

- I When the female plants of dioica were fertilized with pollen from alba all the plants were female. Correns concludes from this result that the diocious condition (with only one sex on one plant) dominates the monocious condition; and since the resulting plants are always of the same sex, the eggs through which the diocious condition was introduced must have been all of one kind—female producing.
- 2 The normal combination of B. dioica ? X dioica & produces females and males in equal numbers. The result shows

that the egg cell of dioica fertilized by pollen from a male plant of the same species produces two kinds of offspring. Combining this result with the first experiment (1) it is certain that the egg cells have a definite sex-tendency, but that the product of their fertilization is not necessarily of the same sex; otherwise all the offspring in both experiments must have been female.

3 When Bryonia alba Q was crossed with B. dioica A half of the offspring were male and half female. Again it is evident that the monœcious condition (males and females on the same individual) is recessive to the diœcious condition. It follows that the decision in regard to sex rests with the male cells. The male germ cells of B. dioica cannot be all of the same sex, for the offspring would then be of one sex only, because it is certain that the female germ cells of B. alba are all alike.

Correns' scheme may be summed up by saying that the male is heterozygous, i. e., it contains both the male and the female tendencies; when the spermatozoa are produced there is a separation of the sex characters and spermatozoa with male and female tendencies result; the female is homozygous and all of her eggs have the female tendency; when a female egg is fertilized by a female sperm a homozygous female results; when a female egg is fertilized by a male sperm a heterozygous male results owing to the dominance of the male character.

Correns' conclusion agrees with that in the insects in so far as two kinds of sperm are assumed that determine the sex of the offspring, but the results are not in harmony with the facts for the phylloxerans, aphids, hydatina, daphnians and some other parthenogenetic forms, because here the fertilized egg produces a female which must therefore be dominant since from it both males and females are produced later. Appreciating the difficulty in the case of the bee, Correns tried to escape from the position to which his view leads him if applied here by assuming that there are two kinds of eggs—male (parthenogenetic) and female (sexual) eggs. Even if it be granted that Dzierzon's theory for the bee is not conclusively proven, yet the facts seem almost overwhelmingly in favor of that interpretation.

In connection with Mendelism and sex, Bateson, Punnett,

Doncaster, Raynor and Durham have brought forward some astonishing facts connected with the assumption of certain characteristics by one or the other sex and their transmission. The evidence is too complicated to go into here but the assumptions necessary to account for these results throw important light on sex as determined by one or by the other parent. Experiments along these lines bid fair to give a further insight into the problem.

Doncaster based his first interpretation of the facts for Abraxas on the assumption that each sex gives off male and female bearing gametes, and on the further assumption that in the first generation there is a coupling so that the male ova bear the A. grossulariata character and the female ova the A. lacticular character; further that in the gametes of the male there is no coupling, that after fertilization dominance attaches to the sex brought in by the ovum.

Bateson and Punnett have simplified this hypothesis and explained the facts by two (three) assumptions; (1) that the female is heterozygous for sex, the male homozygous; (2) that femaleness is dominant to maleness; (3) that in the first generation whenever the two dominants, femaleness and the grossulariata coexist there is a repulsion between them so that each cell gets one or the other of these factors, not both. Here the assumption made for the insects on the basis of primordia determining sex is reversed, the female is heterozygous in the moth according to Punnett and Bateson, and the male, according to the McClung-Stevens-Wilson hypothesis. Wilson asks, apropos of Bateson's and Punnett's view, if the male is homozygous what is the meanig of the formation of two kinds of spermatozoa in many insects? In the case of the bee the contradiction is even more inexplicable without a further assumption. One escape would be to deny that the accessory chromosome, which alone gives a difference in the two kinds of sperm, has anything to do with sex determination, yet a definite relation between that chromosome and sex has been demonstrated and it is difficult to believe that this constancy has no relation to sex determination. Another way out of the dilemma would be to assume that in some cases the discriminating factor lies in the male and in other cases in the female. Here the facts for Phylloxerans furnish the data to support such a view. The parthenogenetic egg contains the possibilities of producing both sexes. In Mendelian language the cgg is heterozygous. In the male on the other hand we also find two kinds of sperm, one of which becomes functional. This functional sperm is that which corresponds obviously to the female-producing sperm of other insects, since it carries the accessories. It is significant therefore to find the corresponding male-producing sperm nonfunctional. The case shows how either the egg or the sperm may contain the discriminating factors. It is interesting to note that neither Stevens nor Dederer has found an accessory present in the spermatogenesis of Lepidoptera. It must exist in the female, if an unpaired chromosome occurs in this group.

On a quantitative interpretation, the male or the female is "heterozygous" in a sense different from that in which this term is generally used in Mendelian parlance. Heterozygous does not mean that the female contains the male and female "unit characters" that are separated in the eggs, but that a differential factor exists—an extra chromosome that is neither a male nor a female "unit character." If it is present two possibilities may be realized, if absent only one, but in either case the material of the egg has always a dual potentiality that is realized in sex; therefore all eggs and all sperm are capable of producing both sexes if the proper conditions for their realization are supplied. It is especially this conception of the problem that seems to me very important and which is liable to become obscured when the Mendelian term heterozygous is applied to sex. How far this same conception may be applied wherever alternative conditions exist in Mendelian inheritance remains for the future to decide. It is not, I think, without advantages, even when applied to this broader aspect of the problem of discontinuous inheritance.

One of the first attempts to apply Mendel's Law to sex was that of Castle in 1903.<sup>19</sup> He assumed two kinds of spermatozoa, male and female, and two kinds of eggs, male and female: The male sperm can only fertilize a female egg, and a female sperm can only fertilize a male egg. The separation of male from female qualities was supposed to take place at the second reduction divi-

<sup>&</sup>lt;sup>19</sup> Strasburger and Bateson had previously suggested the possibility of such an application, and in fact Mendel's letters shows that he had considered also this view as a possibility.

sion, in both cases. On the Mendelian scheme any sperm may fertilize any egg, but if this applied to sex, as Castle points out, there would be three, not two sorts of individuals, but selective fertilization while avoiding this difficulty runs foul of another one, because all the fertilized eggs will be sex hybrids, i.e., all alike each having resulted from the combination of male and female. In other words, while the theory accounts for the combination of male and female characters in every individual, it fails to account for those factors that determine sex, i. e., the sex of the individual that arises from the egg. It is this problem that is the primary requirement of a theory of sex determination. It was obviously impossible at that time to account for the sex of the individual by referring the results to the egg alone or to the sperm alone, for, besides being inconsistent with some of the views adopted for special cases, this assumption would be directly opposed to the Mendelian theory of heredity which assumes that the dominance or recessiveness is due to the intrinsic properties of each character and does not depend on whether it is at the time carried by the sperm or the egg.

Castle argues that "the strongest evidence of the latency of each sex in the other is afforded by the transmission through one sex of the characters of the other. Thus, as Darwin states when the domestic cock is crossed with the hen pheasant, the male offspring have the secondary sexual characters of the male pheasant; these, manifestly, must have been inherited through the female pheasant." There is possibly a fallacy in this argument. The facts only show however that the secondary sexual characters of plumage, etc., of the pheasant are transmitted as dominant characters to the hybrids both male and female.

# SEX DETERMINATION AS THE RESULT OF A QUANTITATIVE RELATION

In attempting to sum up in 1903 the evidence, then recent, as to the factors of sex determination, I pointed out that while the newer facts showed that sex was early determined in the germ cells, they did not warrant the conclusion that "the male and female primordia" in the germ cells are separated, but that at first

neither sex alternative is realized, and that the conditions internal or external to which the germ cells are exposed determine which one comes to the front. Later in 1905-1906 I argued that the determinative conditions are found within rather than without the germ cells, and in the case of the bee the determinative factor seemed to depend on whether one or two nuclei made up the segmentation nucleus. If the egg was fertilized the presence of two nuclei determined that the female condition evolved; if the egg was unfertilized, the single egg nuclei determined that the male condition developed. In the case of the hybrid gynandromorphs described by Toyama an analysis of the results (1907) seemed to show very clearly that a single nucleus derived from a male sperm likewise produced male parts. This case furnished strong evidence in favor of the view that the results are purely quantitative, and are not the outcome of maleness or femaleness, as such, attached to the egg or sperm.

This same point of view advanced for a special case was expanded two years later (1907) in my Experimental Zoölogy into a general theory of sex determination for those forms in which the two sexes had been found to contain more or less chromatin, especially for insects with an accessory, and for cases like the bee in which one nucleus stands for the male, two for the female. At that time I tried to lay special emphasis on the point that the male and the female condition represent alternative possibilities of the protoplasm, which one being realized depending on a quantitative factor in the cases under discussion. In other words that there is not a "segregation" of male from female in the gametes, but some other process which leads to the development of one or the other sex. This point of view looks upon maleness and femaleness as inherent properties of the protoplasm, alternative as to the nature of their development. Special factors condition sex in the sense that they determine sex, but are not sex "determinants" in the Weissmannian sense.

In the light of the most recent results we face the further question as to whether these quantitative factors are only more or less chromatin or more or less of a particular kind of chromatin residing in special chromosomes. An answer to this question

involves the whole problem of the nature of the chromosomes themselves—a question about which we know at present almost nothing. If the size differences of the chromosomes means that they are qualitatively different, then the probable answer is that the quantitative difference depends on more of a particular kind of chromatin. But if the size differences of the chromosomes has only a genetic meaning and they are qualitatively identical or very similar, the probable answer is that the result is merely more chromatin.

The case of Acholla multispinosa described by Payne, in which the single Y-element is quantitatively greater than the five chromosomes that form the X group (the mates of Y), may seem to be demonstrative in favor of the view that the result is not quantitative but qualitative. But even here we do not know whether five separate chromosomes might not be more active than the same material contained in one chromosome. Tempting as it is to assume that the quantitative effects are due to the number of the sex chromosomes qualitatively different from the others, the evidence is still too imperfect to decide so important a question. We can safely leave the solution to the future, nevertheless this case of Acholla is extremely important. The single large chromosome is confined to the male line. It seems arbitrary to ignore its function as a male-producing factor in development. The evidence in favor of this view is at least as great as that for the accessory chromosome in other insects, and the main argument for ignoring it is that it does not fit in well with theories based on the accessories alone.

In a recent analysis of the problem Castle proposes a view that has certain points of resemblance to a quantitative hypothesis, and at the same time attempts to make sex inheritance a phase of Mendelism. "The female is the male condition plus a distinct unit character Mendelian in heredity." In support of this view Castle points out how the case of Abraxas can be accounted for on the basis of the special assumptions made by Bateson and Punnett. The essence of Castle's position is that maleness and femaleness are not allelomorphic, but there exists a unit character whose presence produces a female, its absence a male. A further analysis of the

Abraxas case along these lines shows that Wilson's assumption, based on the chromosomal evidence, that two X's produce the conditions leading to femaleness, one X produces the conditions leading to maleness will not explain the results, while the assumption of one X for femaleness and no X for maleness fits in with the observed relations. This interpretation has the advantage of giving a simple explanation for Abraxas. In fact it is little more than the hypothesis of Bateson and Punnett made more general in the sense that it assumes a unit character that determines femaleness, but for this very reason it introduces an interpretation of sex that is extremely hypothetical. For, the unit character is no longer simply a quantitative factor but a special element that has the power of turning maleness into femaleness. It is an entirely imaginary factor and lacks observational evidence in its sup-It leads to further assumptions in regard to the secondary sexual characters in the male, supposed to be absent in the female. These are referred to another unit character and it is suggested that this is the Y-element of Wilson. characters should be absent when Y is absent, which appears not to be the case. In so far as the view rests on a quantitative conception of sex determination it contains elements that are in harmony with the views here discussed, but in so far as it calls for a Mendelian element whose presence turns maleness into femaleness it meets with serious difficulties and must make further hypothetical assumptions for its support. One of its more serious drawbacks we have already referred to, namely that postulating male and female gametes it assumes that they conjugate without regard to their condition as sex-bearers, male fertilizing male at times, etc. This difficulty might be met by assuming that the unit character for sex (sporophyte) has nothing to do with the conjugation properties of the gametes and the signs male and female are used only as symbols for the presence and absence of this differential character. But such a view ignores the conjugating element itself whose signs cannot be explained on the assumption, here adopted, of their determination by their association with the sex element.

We owe to Wilson the most complete analysis of the present evidence relating to sex determination. His conclusion is based

on the evidence that "half the spermatozoa are characterized by the presence of a special nuclear element which I shall call the 'X-element' while the other half fail to receive this element." In the simplest case the X-element is a single chromosome. In other cases the X-element is the larger of a pair of chromosomes—the smaller called the Y-element. In still other cases the X-element may be represented by two or more chromosomes. Comparison of the somatic number of chromosomes in male and female shows that the X-element (whether a single chromosome or more than one) "is present as a single unit in the male while in the female it is doubled."

Wilson provisionally formulates the following theory. "Males are produced from zygotes that contain but a single X-element; females from those that contain two such elements." In ordinary sexual reproduction all the fertilized eggs should after maturation bear the male tendency because one X-element is left in the egg after reduction. If capable of parthenogenesis with the reduced or haploid number of chromosomes, such eggs should produce males, as appears to be actually the case in the bees and ants. If fertilized by a spermatozoon that lacks the X-element, the egg still produces a male for the same reason. If fertilized by a spermatozoon that contains the X-element, the egg produces a female because of the introduction not of a dominant "female tendency" but of a second X-element.

Wilson points out that there may exist also other factors still unknown and calls attention to the formation of the asexual spores in mosses where there occurs an "apparent disjunction of the sexual tendencies" since these spores contain the reduced number of chromosomes. Nevertheless "we are led to suspect.... from the facts known in animals that the male-producing spores may be characterized by the absence of some element that is present in the female-producing ones." This difficulty is now considerably lightened by the discovery of Baltzer (see below) that in the sea urchin there may exist a pair of chromosomes of unequal sizes associated with sex. If such exist in the mosses their separation at reduction might lead to the formation of two kinds of spores, even if both have the same number of chromosomes. The question,

after all, is one of relation, and not one of absolute numbers. In any cycle there may appear a stage when the separation of the factors that determine sex is brought about and in each species this relation may have become adapted to the presence or absence of certain quantitative or qualitative factors. It is not, therefore, for all cases a question of the haploid or the diploid numbers, or of one or two X's, or of one X or no X, but in each particular case certain factors turn the scale one way or the other.

Boveri has recently described some observations of one of his students, Baltzer, on the egg of the sea urchin showing that two kinds of eggs exist. Half of the eggs have two hook-shaped chromosomes the other eggs have one hook and a homologous rod-shaped chromosome. All the sperm contain a hook and a rod-shaped chromosome. Sex is determined by the egg; in the sense that the egg with the particular hook-shaped chromosome, fertilized by a sperm, gives a female with the rod and the hook-shaped chromosomes as a pair; the egg with the rod-shaped chromosome fertilized by a sperm gives a male with a pair of rods. The result is due in Boveri's opinion to the greater activity of that fertilized egg and its derivatives that contains the rod and the hook-shaped chromosomes, owing to the greater volume of this combination, as compared with the activity of an egg containing the two hook-shaped chromosomes. To the "sex chromosomes" is ascribed no different sex tendencies, but the result is due to greater activation.

#### **GYNANDROMORPHISM**

The appearance of male and female parts in the same individual has been frequently described especially for the insects. I have offered elsewhere a suggestion as to how this condition may arise. It remains only to bring this suggestion in line with the present points of view.

If sex-determination be looked upon as a quantitative factor only, gynandromorphism in such forms as the bee may be accounted for as follows: Two (or more) sperms entering the egg one only fuses with the female pronucleus. Their combined

product gives rise to female parts; the other sperm (or sperms) also developing, give rise to male parts. If in the bee, as in the phylloxerans, the sperms are all of one class, no such complications arise in these cases as might theoretically arise if gynandromorphism should appear in a species having two kinds of sperm, one with, the other without, an accessory. Here two categories of cases appear. If any egg can be fertilized by any sperm, i. e., no selective fertilization occurs, then, should the female-producing sperm enter and fuse with the egg pronucleus, the resulting parts would be female—if another such sperm also entered at the same time, but did not fuse, it would give rise to male parts. Should this other sperm be a "male-producing sperm," so called, it might either produce male parts for all we know to the contrary, although it has no accessory, or else it might not produce normal development of any kind.

On the theory of selective fertilization the following scheme would give the results:

If a female egg carrying the female determinant be entered by more than one sperm carrying the male determinant and one combines with the nucleus, the parts that result from this combination will become female (since the female determinant dominates) the other sperms can produce male parts, hence an hermaphrodite results. But if a male egg (carrying the male determinant) be entered by more than one female sperm carrying no sex determinant, the derivatives of the combined nucleus will produce a female and that derived from the male sperm will produce male parts.

There is still another way in which gynandromorphism may be regarded. If one sex is heterozygous the simultaneous but independent development of dominant and recessive characters would produce gynandromorphism; which could occur only in that sex that is heterozygous. Should both sexes produce gynandromorphs, the result would mean either that both sexes are heterozygous; or that abnormal fertilization, as explained above, is responsible for the effects.

The following evidence on the effects of castration have a bearing on the problem of sex.

It has been shown for the mammals and birds especially that

removal of the ovaries at an early age causes the development of "male" characters (somatic). The result shows unmistakably that the female in these groups has the capacity to produce both secondary sexual characters. It is also sometimes claimed that castration causes the male to develop the characters of the female, and in favor of this view the retention of the high voice by eunuchs and the absence of a beard have been supposed to indicate an approach to the female. It is, however, evident that these are juvenile characters, and that castration has only suppressed the complete development of the male. Other cases have been cited that seem to their authors to show an approach to the female type, but the evidence is not as convincing as that in the case of the female. In the insects there seems to be no correlation between the development of the secondary sexual characters and the sexual organs, so that we cannot test the nature of the individuals in this way.

In the crabs the evidence is as follows:

In the crustacea (Carcinus, Inachus, Stenorynchus) there are certain facts according to Geoffrey Smith connected with parasitic castration that have been interpreted to mean, that the male assumes the secondary characters of the female when the testes are Instead of the narrow abdomen of the male, the infected males have a broad abdomen more like that of the female and the copulatory abdominal appendages of the male are wanting. Instead the corresponding parts are biramous like those of the female. It may appear, therefore, that the male is heterozygous and produces the female character when the male organ is suppressed. But the facts might possibly be interpreted in another way. The broad abdomen of the castrated male might be considered to correspond to the juvenile state. The only external structure cited by Smith that might seem to indicate that the characters of the castrated males are female rather than juvenile ones is the presence of hairs on the abdominal appendages of Inachus, absent in the young crab, but present in the adult female. Such evidence would not in itself be conclusive, since the presence of hairs may be due to increase in size or to a later moult rather than to latent female characters. The claws of the male of Inachus also differ from those of the female, but here again the

claws of the castrated male seem to be in most respects as like the juvenile claws as they are like those of the female.

A number of infected crabs showed a condition of hermaphroditism, having, for example, the broad abdomen but with copulatory styles on it. Smith concludes "that the male sex and probably the male sex alone can be so radically modified in its sexual nature as to assume a perfect external hermaphroditism." If, on the contrary, we assumed that we have here not hermaphroditism but an imperfect development of male characters combined with the juvenile condition, we might offer a plausible explanation of the facts.

On the other hand two considerations of great weight point in the opposite direction. Smith found several cases in which the parasite had left the host and the latter had recovered. In such cases a perfect hermaphroditic gland regenerated that produced both spermatozoa and ova. One individual had developed both oviducts and vasa deferentia. This fact alone would demonstrate that the male crab is in reality heterozygous.

Infected females never show the male characters, as is the case in castrated vertebrates, even when the ovary is completely removed. This might seem to show that in the crabs the female is homozygous, at least if the evidence for the vertebrates is logically applied to the crabs. There is, however a curious point here, seldom, if ever, noticed. Why should one expect, if both sexes have dual possibilities, that the destruction of the ovary causes the male characters to appear and the destruction of the testes the female? I see no logical grounds for such an expectation.

## THEORETICAL DISCUSSION

In the unsettled condition of the evidence it is obvious that the problem of sex-determination has by no means reached a satisfactory solution. It is equally clear that a large amount of data is rapidly accumulating that promises to furnish an insight into the conditions that regulate sex. The evidence furnished by the phylloxerans shows that the parthenogenetic egg of the female has the dual capacity of producing either males or females. There

is little to show that these parthenogenetic females, prior to the reduction divisions, differ essentially from ordinary females. It is a fair inference perhaps, that in some cases at least the sexual female may contain dual possibilities.

The spermatogenesis of the phylloxerans shows that two classes of sperms are produced and it is significant, I think, to find that the class of functional sperm corresponds to that class in other insects that is "female-producing." This means only that this combination gives rise to a female, not necessarily that the sperm carry female determinants.

In fact the analysis of the results if based on the assumption of male and female determinants demands that the "female-producing sperm" carry the male determinant. Such sperm would introduce into the sexual egg those elements which, after being carried through one or more parthenogenetic generations, make possible the production of males. The only clue as to what these elements might be is found in the accessory chromosomes. The following situation then develops: The accessories left in the sexual egg must be the female accessories; the functional sperm brings in the male accessories. It follows that the male-producing parthenogenetic egg must have eliminated its female accessories in order to produce a male. There are then two (pairs of) female-producing chromosomes and one (pair of) male-producing. At the time of extrusion of the polar bodies in the male egg of the phylloxerans, the two female accessories must always be thrown out and the two male accessories retained. Conversely for the sexual egg. Extending the same reasoning to other insects with accessories it follows that the "female-producing" sperm contain the male accessories. Such sperm must fertilize eggs containing the female accessories and produce the heterozygous female. The sperm without the accessory must fertilize an egg containing the male accessories and produce the homozygous males. This means selective fertilization.

Such seems to me to be at present the logical outcome of the assumption that the accessories are sex-determinants if their determination is a qualitative process.

Several objections to this view have been already urged and

need not be repeated here in extenso. The most serious one is that although the hypothesis is ostensibly based on the presence of certain chromosomes which are assumed to be male and female determining respectively, yet to these chromosomes, which are to all appearances identical are ascribed exactly opposite functions; and in order to make the scheme workable it must further be assumed that selective fertilization occurs—a process still unproven.

For these and other reasons, sufficiently discussed in the preceding pages, it seems more probable that the relation of these chromosomes to sex may be a quantitative one. This interpretation I urged especially two years ago in my general treatment of the problem of sex in my Experimental Zoölogy. The same view I had previously argued in the special case of the bee, and in the case of gynandromorphism in the bee and silk-worm moth. shall repeat here, therefore, in conclusion, the essential part of the general argument advanced in the Experimental Zoölogy. It was pointed out that we can explain the cases in which an accessory is present on the assumption that the sperm with the accessory brings more chromatin into the egg than does the sperm without that chromosome. In the bee the result is due to two nuclei leading to the formation of a female, one to the formation of a male. pointed out that on this view sex is not laid down in the egg or sperm but is determined later by quantitative relations that appear as a result of certain combinations. The objection to this view is found in those cases where no accessories exist, but even here the recent results of Baltzer show that differences between the pair of sex chromosomes may still be present. It remains to be discovered whether this relation holds in general or not. obviously go beyond the evidence to assign the determination of sex to differences in the chromosomes in those cases where no difference has been observed. The remarkable cases here described for Phylloxera fallax show that the determination of male and female lines may take place in the presence of all the chromosomes. We have in this case strong evidence in favor of the view that other processes than the number of chromosomes may initiate changes that ultimately lead to the production of one or the other kind of individuals. For obviously it is as important to discover what

factors determine that one winged migrant produces only female eggs and another only male eggs, as it is to determine maleness and femaleness itself.

"The average equality of males and females indicates, I think, that external conditions do not regulate the results but that some internal physiological mechanism exists that determines the sex. Of course this statement does not exclude the possibility that external influences may determine that the internal mechanism shall become active in one way or another, as seen in the cyclical modes of reproduction. This physiological mechanism does not involve the separation of male and female elements or units in the egg and sperm but only involves the production of those conditions that determine whether one or the other sex will develop." In the group of insects these conditions seem to be connected with the accessory chromosomes as quantitative factors. Their separation does not involve in itself the separation of maleness from femaleness, but the separation of one special chromosome in such a way that two classes of individuals result after fertilization. Possibly the same conditions may be brought about in other ways in other animals.

"It seems not improbable that this regulation is different in different species, and that therefore it is futile to search for any principle of sex determination that is universal for all species with separate sexes; for while the fundamental internal change that stands for the male or the female condition may be the same in all uni-sexual forms, the factor that determines which of the alternative states is realized may be very different in different species." this point of view justifies itself, the problem of sex determination resolves itself into a search for those factors that turn the balance towards one or the other of the two possible alternatives. Let us not forget that while approximate equality of male and female is for many species the general result, it is not true for the output of special individuals, and also not true for certain species in which an excess of one or the other sex exists. Nowhere is this better seen than in the offspring of the same stem-mother of P. caryæcaulis where all may be male producers or all female producers.

#### CONCLUSION

The quantitative interpretation of sex-determination is only the first rough approximation, I think, to a solution. It is valuable because it is supported by a large number of observations in which such a quantitative difference is apparent. But there is nothing in these facts that shows that the effects are directly quantitative rather than that observable quantitative differences accompany, or follow in some cases, more profound changes. should not be forgotten, moreover, that the quantitative differences have been found in only a relatively small number of cases; that they are absent in others, and that in such cases as the male and female eggs of phylloxerans where size differences exist in the egg as a whole—differences that appear directly associated with the formation of the male and the female—the differences originate in the presence of the entire number of chromosomes. The loss of certain chromosomes from the male egg appears to follow, not to precede the size relation. These considerations may arouse a suspicion at least that it is not chance that determines to which pole the accessory chromosome moves when it is present, but that its pole is predetermined. At least the facts here described for the phylloxerans and aphids may be urged in support of this view. If such differences already exists between the two daughter sperms these differences may subsequently be as intimately connected with the sex of the embryo as the accessory itself. The accessory may follow sex or be associated with other differences that determine sex rather than be its sole cause.

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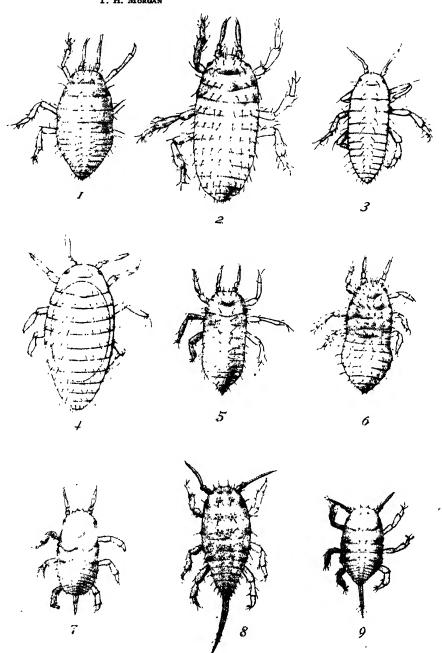
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#### DESCRIPTION OF PLATE I

- Fig. 1 Male of P. fallax (somewhat compressed).
- Fig. 2 Female of P. fallax.
- Fig. 3 Male of P. fallax (preserved specimen).
- Fig. 4 Female of P. fallax (preserved specimen).
- Fig. 5 Male of P. caryæcaulis.
- Fig. 6 Female of P. caryæcaulis.
- Fig. 7 Sexual (?) form of P. subelliptica.
- Fig. 8 Sexual (?) form of P. caryæglobuli.
- Fig. 9 Sexual (?) form of P. depressa.



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Riccioli del.

# FACTORS OF FORM REGULATION IN HARENACTIS ATTENUATA

## III REGULATION IN "RINGS"

BY

#### C. M. CHILD

WITH THIRTY-ONE FIGURES

#### I EXPERIMENTAL DATA

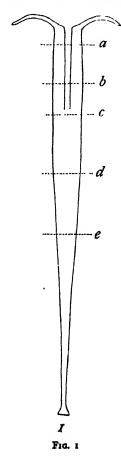
# I The Formation and Structure of Rings

Certain methods of formation of the peculiar structures, which for lack of a better name I have termed "rings." were briefly described in first paper of this series (Child 09b), as illustrations of the remarkable results which the wound-reaction may bring about under certain conditions.

The basis of the ring is a rather short cylindrical piece cut from any region of the body distal to the attenuated portion. Pieces between any two of the consecutive levels of section indicated in Fig. 1, or even somewhat shorter pieces than these would serve under certain conditions for the formation of rings.

The ring is formed by the union of the oral with the aboral cut surface of the body-wall, and such union almost never occurs except perhaps in very short pieces, unless longitudinal wounds exist on the mesenteries, whose contraction approximates the oral and aboral cut surfaces of the piece. These longitudinal wounds may be produced in various ways; for example, in a piece such as ab, Fig. 1, we may simply remove the esophagus by cutting the mesenteries which hold it in place, or in subesophageal pieces such as cd or de, Fig. 1, we may remove the longitudinal muscles before the piece closes. These operations are not difficult except for the fact that the very strong contraction of the body-wall under the continued stimulation often results in tears or other injuries which

interfere with the result. With the aid of an anæsthetic this operation could undoubtedly be performed very easily, but I have found this unnecessary since a very simple means of attaining the desired result is available for all pieces except those in the æsophageal region. The muscles and other mesenterial organs below the



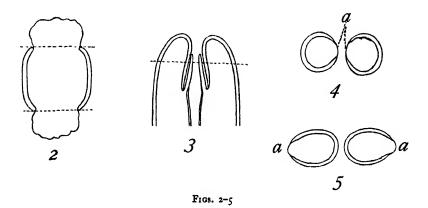
cesophagus are bulky and the contraction following a cut in this region almost always results in forcing a considerable mass of these organs out of the opening. This is especially the case in short pieces which contract more strongly than long pieces, doubtless because the whole piece is stimulated from both cut surfaces.

In such pieces the extruded mass of mesenteries and muscles can readily be cut off (Fig. 2). The operation of removing this mass often stimulates the piece to further contraction and extrusion, so that a second and sometimes a third mass may be removed. In this manner exactly the same result may be accomplished more or less completely as by the method of dissection, and much more readily. It is usually unnecessary to remove the free borders of all mesenteries throughout the whole length of the piece in order to produce rings. If the longitudinal wounds are sufficient to approximate the oral and aboral cut edges in some degree, they usually meet, especially if the length of the piece in its contracted condition is much less than its diameter. In such pieces the shape alone determines that union of oral and aboral cut surfaces with each other rather than the usual method of closure shall occur. simply because when the oral and aboral ends of the body-wall roll inward after the operation they are not long enough to form a closure across the axis in the usual manner and contraction continues until oral meets with aboral cut surface and union occurs. In general the shorter a piece the more likely it is to form a ring. But since in the very short pieces regulation often does not proceed beyond the formation of new tissue along the line of union, I have found it preferable to use longer pieces and to induce this method of closure by the operations above described.

There is, however, still another very simple method for producing rings, one which requires no second operation. This consists in the removal of the distal ends of invaginated individuals. This method is illustrated by Fig. 3, the broken line indicating the level of section. By this operation a short piece is isolated which contains none of the longitudinal muscles or marginal mesenterial organs and at the same time each mesentery has a cut edge throughout the length of the piece. Consequently the conditions for the formation of rings are established at once in these pieces and as a matter of fact such pieces form rings in practically every case, the only exceptions to the rule being cases in which other disturbing factors are involved.

The union between oral and aboral cut surfaces in the rings occurs in the manner indicated in Fig. 4, a diagrammatic section,

the line of union being at a. Both the oral and aboral parts of the body-wall near the cut undergo more or less differentiation and growth and become much thinner as in other cases of wound closure, so that the region of union is marked by a band of transparent new tissue, which in section is much thinner than other parts of the body-wall (Fig. 4).



By this method of closure the enteric cavity loses all connection with the exterior except such as may occur through the cinclides. Since the original oral end unites with the aboral end in such cases the terms oral and aboral lose their significance so far as the piece as a whole is concerned, but it will be convenient in the following description to designate as oral and aboral respectively, those parts of the body-wall which originally formed the oral and aboral ends of the piece. Thus in Fig. 4 the oral end lies just above the line of union at a and the aboral end just below.

After closure the rings become more or less distended by the entrance of water through the body-wall, though in all cases the distension is, as in esophageal pieces, far below that of the normal animal. They also perform certain rather remarkable movements which result in most cases in changing the position of the region of union so that it comes to lie somewhere near the equator on the outside of the ring (Fig. 5, a, a) instead of at the equator facing the central opening (Fig. 4, a) as originally. This movement may be described as a revolution of the tissues about a circular axis situ-

ated in the enteric cavity of the ring. It undoubtedly results from differences in the degree of contraction of different parts of the body-wall, and since in most cases it accomplishes a more or less definite and characteristic result, I am inclined to believe that it is really an attempt at orientation, though of course anything like normal orientation is impossible. At any rate the fact remains that most of the pieces assume the position indicated in Fig. 5 and this position is usually retained, although stimulation may bring about a temporary return to the original position or may change the position of the region of union to the upper or lower surface. Such changes are usually followed by a return to the position of Fig. 5.

# 2 The Course of Restitution in Rings

Restitution in these rings consists, briefly stated, in the formation of single tentacles, of irregular groups of tentacles, or of small discs with complete circles of tentacles varying in number. The tentacles may form on both sides of the line of union, i. e., they may be both oral and aboral or they may appear wholly on the oral side of the line of union. In the latter case discs with complete circles of tentacles are usually formed, though these sometimes appear when the tentacles are of both oral and aboral origin. The number of tentacles or groups formed varies greatly in different cases and is apparently indefinite.

It will be convenient to consider the data under two heads, viz: those cases in which tentacles are formed on both sides of the line of union and those in which they appear only on one side. The following figures of the rings are on a larger scale than preceding figures. In all cases where the line of union is visible in the aspect shown in the figure its position is approximately indicated by a dotted line. In the specimens the region of union is not sharply marked off from other regions but is distinguishable as a band of transparent new tissue of varying width, in the formation of which both the oral and aboral end of the body-wall have taken part. The line of union indicated by the dotted line in the figures represents as nearly as possible the middle of this band. In many of the figures the tentacle-groups are represented with the discs-

turned more or less toward the observer in order to show more clearly the arrangement of tentacles.

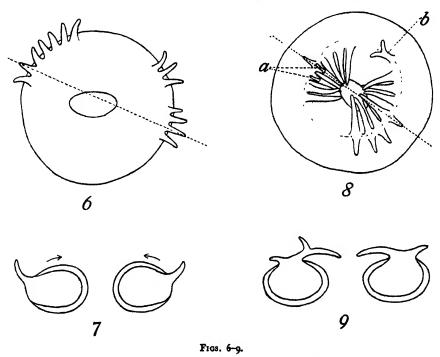
#### Cases with Tentacles on Both Sides of the Line of Union

The following cases are selected from among a much larger number as illustrations of the results in such rings. No two individual cases are alike but the cases described are sufficient to show the principal features.

I Figs. 6 to 9. Piece from the region just proximal to the cesophagus: after section some portions of the mesenterial organs extruded through cut ends and removed, but a considerable bulk of these organs remained. Piece closed by union of oral and aboral ends, forming a ring. Tentacles began to appear eight or nine days after section. Fig. 6 shows a stage eleven days after section. The line of union between oral and aboral ends is at the equator outside and is therefore not visible. The tentacles present are all developing from the original oral end. Fig. 7 represents a section in the plane of the broken line in Fig. 6; here the thin new and redifferentiated tissue at the region of union and the position of the tentacles with reference to it are indicated.

Fig. 8 shows the piece seven days later. Fig. 9 is a section of the stage of Fig. 8 in the plane of the broken line. A change in position of the region of union has occurred in consequence of the revolution of the tissues of the ring about a circular axis as above described, and the region of union now lies at what might be called one of the poles of the ring, i. e., half way between the equatorial region on the periphery and that in the central opening. The number of oral tentacles is the same as before but their length has increased and the change in position of the oral end has apparently altered their relation to other parts. Comparison of Figs. 7 and 9 shows the character of this change. The revolution has occurred in the direction of the arrows in Fig. 7 and has brought the region of union into the position shown in Fig. 9. But the most interesting feature of Fig. 8 is the appearance of tentacles on the aboral side of the line of union. Five aboral tentacles are present at this stage; two of the oral tentacles bear branches near

their bases, which correspond in direction of growth to aboral tentacles (a, Fig. 8, also the left half of Fig. 9), and finally one tentacle, which is apparently oral-aboral, has appeared (b, Fig. 8): it arises at the line of union and is evidently formed by the fusion of two tentacles one of which was oral, the other aboral. In the later history of this piece the aboral tentacles increased somewhat in length, but within a week after the stage of Fig. 8



decrease in the distension became marked and all tentacles began to atrophy at the tips, and gradual collapse and finally death followed.

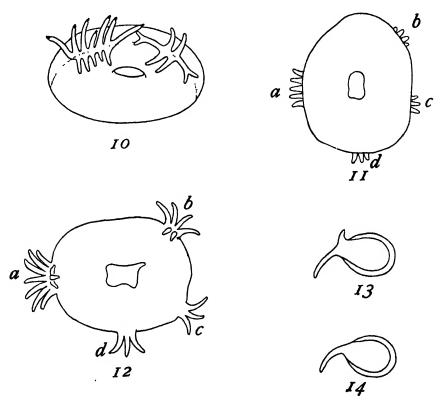
The following are the chief points of interest in this case: tentacles do not appear about the whole circumference, but are grouped; aboral tentacles appear later than oral—except in the single oral-aboral tentacle, where the two parts evidently arose at the same time—and are in all cases opposite oral tentacles, though the aboral are less numerous than the oral tentacles.

II Fig. 10. This piece, like No. 1, is from the region just proximal to the œsophagus. Some portions of the mesenterial organs protruded after section and were removed, but a large part still remains. Fig. 10 shows the piece twenty-five days after section, when the tips of the longer tentacles were just beginning to show atrophy in consequence of the decrease in distension. Only the two groups of tentacles shown in the figure appeared on the piece. Each of the two groups has an equal number of oral and aboral tentacles—five in the one, two in the other—and each has an oral-aboral tentacle at each end. In the group at the right the oral-aboral tentacles are forked, i. e., they began their development as separate oral and aboral tentacles and then fused; in the other group the two tentacles at the ends are simple, and while their position and relation to the other tentacles and the line of union seem to indicate that they are oral-aboral in character, yet they appear to belong more to the oral (in the figure, the upper) side of the line of union than to the aboral, and in the history of the piece their time of appearance and stage development is of much more like the oral than the aboral tentacles.

In this case, as in the preceding, aboral tentacles appear later than oral except in the composite tentacles, and aboral tentacles are always opposite oral tentacles.

III Figs. 11 to 14. As nearly as could be determined, the piece represents approximately the region between the lines d and e in Fig. 1. After section a large part of the mesenterial organs protruded from the cut ends and was removed. In Fig. 11 eight days after section, four groups of tentacles, a, b, c, d, have appeared at the oral end (in Fig. 11 the line of union is at the equator and the oral end with the tentacles is just below it.) Fig. 12 shows the piece six days later, i. e., fourteen days after section. The shape of the piece has changed so that the long axis is at right angles to its previous position. The group a has five more tentacles than before, three of them aboral, and the group a has two aboral tentacles, the groups a and a have merely increased in length. This case differs from both the preceding in that the tentacles of the groups a and a horm almost complete circles: if the tentacles on the aboral side of the line of union were of the

same length as the others these two groups would appear, except for the smaller number of tentacles, like complete discs. Mouth and æsophagus are, however, absent and never appear. Fig. 13 shows a section through the group a and group b is quite similar. Aboral tentacles never appear in the groups c and d, but the three tentacles present form part of a circle. Fig. 14 shows the position

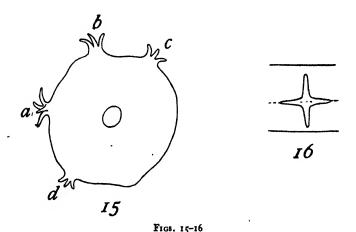


Figs. 10-14

of the middle tentacle of the groups c and d with reference to the region of union. The two lateral tentacles of each of these groups arise nearer the line of union than does the middle tentacle and may perhaps be oral-aboral, though as will appear below it seems probable that in many cases the terms oral and aboral retain only a regional significance in the region of union.

As compared with the preceding cases the most conspicuous feature here is the grouping of the tentacles in circles or partial circles instead of in rows along the two sides of the line of union. This arrangement of the tentacles suggests that there is some approach to the formation of new discs at several points about the margin of the ring. If this is the case a new polarity must be established at each of these points.

This piece never produced any more tentacles; about three weeks, after section decrease in the distension became marked and finally the body-wall became so contracted or reduced that the mesenterial organs burst through the region of union at various points and death soon followed.



IV Figs. 15 and 16. This piece was taken from the region just proximal to the esophagus. Considerable extrusion of mesenterial organs occurred and the extruded parts were cut off, but some portions remained and were included when the piece closed. Fig. 15 shows the piece eleven days after section: four groups of tentacles have appeared, one of them consisting of four tentacles of equal length, forming a complete circle, the other three groups of three tentacles each, forming a partial circle. In the figure the line of union is at the equator and the two lateral tentacles of each group apparently arise from it; in the groups b,

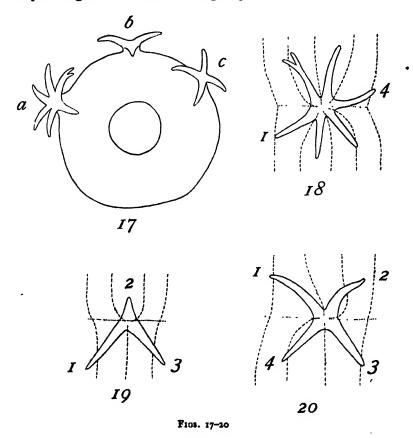
c, and d the middle tentacles arise on one side of the line of union, probably the oral side, if this case is similar to others, though there is no visible distinction between oral and aboral regions in this piece except these tentacles. In group a one tentacle arises on one side of the line of union, one on the other and two from the line of union. Fig. 16 shows this group of tentacles in surface view, the line of union being approximately indicated by the broken line.

No more tentacles were produced and ten days later the piece was almost wholly collapsed and the tentacles were reduced to slight elevations: death occurred a few days later.

This case is not very different from No. III. The only difference of importance is that in the one group where the tentacles form a complete circle they all arose at the same time and are of equal length, although they are partly from the original oral end and partly from the aboral, i. e., in this case the difference in time of appearance of tentacles on the two sides of the line of union, which was apparent in the preceding cases, is not present.

V Figs. 17 to 20. The piece was taken from about the region c, d, Fig. 1: Parts of the mesenterial organs were extruded and cut off, but considerable portions remain. Fig. 17 shows a stage twelve days after section: the line of union is at the equator. Three groups of tentacles have appeared, each forming a complete circle: group a has six tentacles, group b four and group c three. Figs. 18 to 20 are surface views of the three groups and also show the relations between the mesenteries and the tentacles: the line of union is indicated by a dotted line. Discussion of the mesenterial relations is postponed to a later section, but attention may be called here to the radial arrangement of the mesenteries in the regions of tentacle groups and to the fact that tentacles apparently form as readily between the oral end of one mesentery and the aboral end of another as between two oral ends. two lateral tentacles in Fig. 18, the two long tentacles in Fig. 19 and the right lower and left upper tentacle in Fig. 20 arise between oral and aboral ends of mesenteries. These figures are given • merely as illustrations of the mesenterial relations, not because these groups differ in any way from those already described.

all of the preceding cases oral-aboral tentacles occur and in all except No. I and the longer group in No. II the mesenteries acquire a more or less perfect radial arrangement about a point corresponding to the center of the group of tentacles.

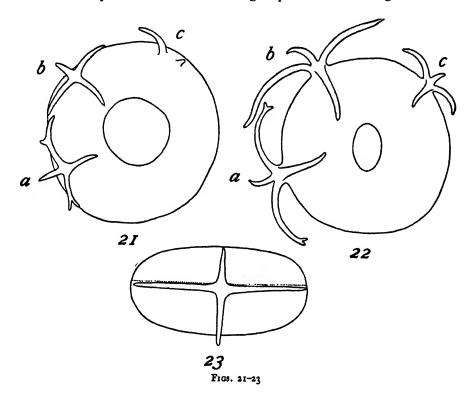


Cases with Tentacles only on the Oral Side of the Line of Union

Most of these cases appear much like the preceding, except as regards the position of the tentacles with respect to the line of union. In these cases none of the tentacles are oral-aboral and none are aboral, but all arise from the original oral end of the bodywall. It is interesting to note, however, that those which lie

nearest the line of union and so nearest the original aboral end often develop less rapidly than the others.

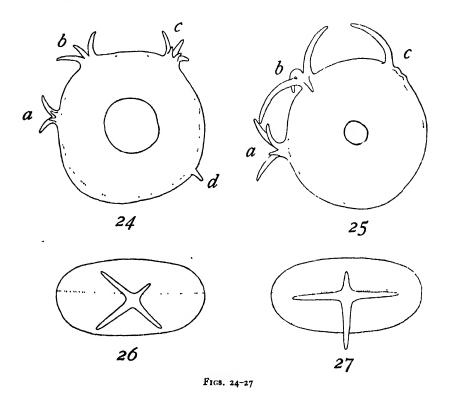
VI Figs. 21 to 23. The piece was from the middle region of the body and repeated extrusion and removal of mesenterial organs preceded closure, only a small part of the organs remaining. Fig. 21 shows a stage eleven days after section: the line of union is at the equator and the tentacle groups are on the original oral



end of the body-wall. Fig. 22 is a stage eight days later. This piece produced longer tentacles than any other observed; the two longest tentacles in group b (Fig. 22) were 10 to 12 mm. in length. Fig. 23 shows the relation of group b to the line of union, the lower side in the figure being the oral, the upper side the aboral. The other groups show similar relations and it should be noted also that in all three groups the tentacle which

is nearest the line of union, i. e., nearest the original aboral end of the body-wall is the shortest tentacle of the group, though it is at the same time the most oral.

This piece remained in good condition for nearly a month, an exceptionally long life for such pieces, before decrease in distension became marked, but after this collapse gradually occurred and death followed.



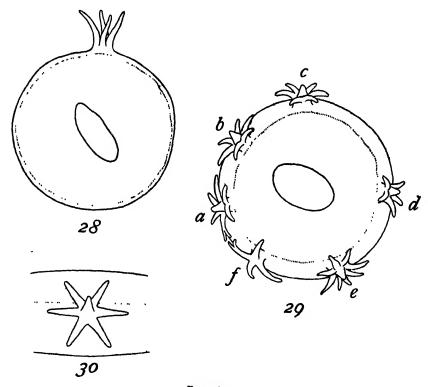
VII Figs. 24 to 27. This piece like No. VI was from the middle region of the body and its mesenterial organs were removed in large part before closure. Fig. 24 shows the piece eleven days after section and Fig. 25 nineteen days. The line of union in both figures is above the equator as is indicated by the dotted line, and the tentacle groups arise just below it, i. e., on the original oral end of the body-wall. In Fig. 24, three groups, a, b and c, and an isolated tentacle at d are present; eight days later (Fig. 25) the tentacle at d and all but one of the tentacles in the group c have disappeared. Figs. 26 and 27 show the relations of groups a and b (Fig. 25) to the line of union. In all the groups the tentacle or tentacles (group a) lying nearest the line of union appear later than the others in the earlier stages. In group a two tentacles are shorter than the others in the earlier stages (Fig. 24) but later one of these attains the same length as the others (Fig. 26).

After twenty-four days this piece was almost completely collapsed and death followed a few days later.

VIII Fig. 28. This piece was also taken from the middle region of the body and a large part of the mesenterial organs was removed before closure. In Fig. 28 nineteen days after section, the line of union is just above the equator and a single group of tentacles appears just below, i. e., on the oral side of it. In this case the four tentacles of the group all appeared at the same time and are all equal in length. No other tentacles appeared and the piece soon began to collapse and degenerate.

IX Figs. 29 and 30. This case is the most remarkable of all observed and is the only one of its kind which I succeeded in obtaining. It was taken from the middle of the body and most of the mesenterial organs were removed before closure. Fig. 29 shows its condition fifteen days after section. The line of union lies above the equator in the figure and just below (oral to) it are six complete circles of tentacles: three of these, a, b and c are alike and consist of six tentacles each; group d possesses only five tentacles, group e eight and group f four. In all the groups the tentacles are arranged symmetrically with respect to a line through the group parallel to the line of union. Fig. 30 shows the arrangement in groups a, b and c and the relation to the line of union. The tentacles all appeared at the same time and are all equal in length.

But the most interesting feature of this case is the presence in the five groups a-e of a conical outgrowth in the middle of the disc. This structure is shorter and more blunt than the tentacles and does not react to contact like the tentacles: its appearance in the same position and form in the five groups indicates that it is not merely some chance irregularity of growth, but rather a structure correlated in some definite way with the others. In the first place it indicates the formation of a region of growth in the middle of the disc. In normal and regulating individuals such a region of growth forms at this point and gives rise to the œsophagus. It



F1G8. 28-30

seems not improbable that this outgrowth may represent the esophageal region, which is evaginated instead of invaginated. But in no case could any trace of an opening be found at the tip of the outgrowth, though such an opening might have been formed if the specimen had lived longer and undergone further growth. If this outgrowth does represent an evaginated esophagus certain factors existing in this case must determine the peculiar method

of growth. Concerning the nature of these only surmise is possible. It is perhaps worth while however, to call attention to one point: from certain of my observations on regulating actinians it has seemed probable to me that the so-called ingrowth of the cosophagus is not actually so much an ingrowth as a growth in the oral direction of the structures about the cosophagus and of the cosophagus itself, i. e., the mouth-opening forms on the disc and growth of all parts in the oral direction brings about the formation of the cosophageal tube, the original mouth-opening being at its aboral end. In the case under consideration almost no growth of the small discs occurs after the formation of the tentacles. If the above suggestions as to the method of cosophageal development are correct the localization of a region of growth where the cosophagus usually forms might perhaps result in an outgrowth as in this case. This suggestion may or may not be correct.

This is the only case in which structures which might possibly represent the œsophagus are formed. The only reason for the presence of such structures in this case and their absence in others which suggests itself is that perhaps in the present case the regulatory tentacle-groups represent something a little nearer to a normal oral end than in the other cases. There are several features which indicate that such may be the case; in the first place the symmetry of the groups and the equal length and simultaneous appearance of the tentacles constitute a marked difference from all other cases except perhaps No. VIII; secondly, examination of these groups showed what seemed to be new mesenteries, radially arranged and apparently quite distinct and separate from the old mesenteries. In some of the cases previously described new mesenteries were apparently present about a part of the circumference of the group but in this case all were apparently new. If these observations are correct it appears probable that the five groups of tentacles under consideration do perhaps represent a somewhat closer approach to normal oral ends than other cases and this difference may determine the establishment of an œsophageal region of growth in these groups and its absence in the others.

After two weeks this ring gradually collapsed and the tentacles

underwent reduction to mere stumps and death occurred a few days later.

#### II DISCUSSION AND ANALYSIS OF DATA

#### I The Nature of the Tentacle Groups

The question as to whether the groups of tentacles which appear on the rings represent more or less close approximations to complete oral ends has already been touched upon in the description of case IX. It seems to me that the general form of these groups, the frequency of a radially symmetrical arrangement of tentacles and mesenteries and the formation of a short column below the tentacles in most cases all indicate that these structures are in reality new oral ends, more or less different from the normal because the conditions under which they arise are more or less widely different from the normal. If this conclusion is correct the formation of these structures involves the establishment of new polarities about the circumference of the ring, and if we can judge from the appearance of the groups of tentacles, such polarities are very evidently established.

The number of tentacles is indefinite, at least up to ten (Fig. 12a) or twelve (Fig. 10). Groups with even numbers of tentacles are much more frequent than groups with odd numbers, even in cases where the tentacles are not equally distributed on the two sides of the line of union (Fig. 12, a and b). This fact is significant, since it suggests at once that the mesenteries in these groups are usually paired as in the normal animal. Moreover, most of the groups with odd numbers of tentacles are the result of the failure of tentacles to appear on the aboral side of the line of union opposite those on the oral side (Fig. 12, groups c and d; Fig. 15, groups b, c and d). Probably these cases are due simply to early cessation of growth in these rings; if conditions favorable to growth continued to exist for a longer time there is little doubt that these cases would become symmetrical with an even number of tentacles like the others. In the first series of these rings, in which the tentacles of each group arise from the two sides of the line of union, only one group with an odd number of tentacles has been observed, viz:

group b in No. V (Fig. 17, b; Fig. 19). In the second series in which tentacles arise wholly on the oral side of the line of union, two groups with odd number of tentacles occur: these are group c in No. VII (Fig. 24) and group d in No. IX (Fig. 29). Evidently then, these cases are not common: they are probably the result of special conditions which determine the delay or inhibition of a tentacle, perhaps in some cases of more than one. In the case shown in Fig. 19, for example, the mesenteries on the two sides of the line of union do not correspond in position because of irregularities in closure, and the number of tentacles is determined by the number of intermesenterial spaces involved in the region of growth. Concerning conditions in the other two cases nothing definite can be said: probably in these also merely incidental factors are concerned.

The cases described were arranged so far as possible to show the gradation from groups of tentacles irregular in number and form (No. I, Fig. 8; No. II, Fig. 10) to the other extreme of symmetrical discs (No. IX, Fig. 29).

The question now arises as to whether these groups of tentacles are to be regarded as cases of heteromorphosis. The cases in the first series (Nos. I to V) in which the tentacles arise in part from what was originally the aboral end of the body-wall are certainly heteromorphosis so far as these aboral tentacles are concerned.

As regards the cases in the second series, where the tentacles are all formed on the oral end of the body-wall, polar heteromorphosis in the usual sense certainly does not occur, since aboral structures do not arise from what was originally the oral end or vice versa. On the other hand, a given region of the oral end of the body-wall which originally represented only one side of the body produced structures characteristic of all sides. Properly speaking this is as truly heteromorphosis as are the polar reversals. In these cases the symmetry of parts of the region involved in the formation of the tentacle groups must have undergone reversal since it produces parts characteristic of the opposite side of the body, i. e., a radial heteromorphosis has occurred in these cases.

In other words in these rings changes of polarity and symmetry occur which lead to the formation, either on both sides or on the

oral side only of the line of union, or more or less complete, usually radially symmetrical groups of tentacles which undoubtedly represent more or less completely oral ends. In the following paragraphs some of the probable or possible factors involved in the localization and development of these structures are considered.

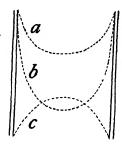
## 2 Localization of the Tentacle Groups Along the Line of Union

The localization of the groups is one of the most striking features of the rings, and the question at once arises as to what factors determine that isolated groups of tentacles, indefinite in number and position, shall be formed rather than a continuous series of tentacles about the oral end. If the oral end can produce tentacles at all why does it not produce them in the usual manner? Manifestly the answer to this question is to be found in some of the conditions of the experiment. The characteristic feature of all these cases is the union of oral and aboral ends about the whole circumference; no free end or terminal region like that from which oral structures usually arise, exists in these pieces. It seems highly probable that the union of oral with aboral end brings about changes in the physiological conditions characteristic of these two regions, since the two ends are in direct organic continuity with each other. If such changes occur they must consist in a decrease in the differences between the two ends and it is probable that the new tissue at the line of union is neither oral nor aboral in its physiological characteristics, or at least that it is less "oral" in character than the new tissue formed by the closure of the oral end in the usual manner. The fact that tentacles do not appear at all in the rings until long after their formation in pieces of the usual sort from the same region indicates that some change, apparently quantitative, has occurred, for so far as morphological conditions are concerned there is no obstacle to the formation of tentacles. Under the usual conditions, i. e., at the oral end of a cylindrical piece the tentacles arise, not directly from the cut surface, but are localized regions of growth and differentiation in a continuous sheet of tissue formed by the closure of the cut ends. The continuous sheet of tissue is present in the rings after closure and there is no apparent reason why a continuous circle of tentacles should not be formed about the circumference of the oral region of the ring, provided the physiological characteristics of this region remain after closure the same as they were before. The only possible inference from the facts as we know them at present seems then to be that the union of oral with aboral end has altered, at least quantitatively, the physiological characteristics of the oral end. Concerning the aboral end the case is not so clear in the earlier stages of restitution, though, as will appear below, the aboral end is apparently altered more than the oral end, since it gives rise in many cases to tentacles.

In short, a decrease, though apparently not an elimination of the original polarity in the piece constitutes the first step in determining what follows, i. e., the establishment of a new polarity, for we cannot doubt that each of the tentacle groups represents the establishment in some degree of a new oral-aboral axis.

We have now to consider how the regions where this new polarity may be established are localized. In the first place conditions are not identical about the whole circumference. When the piece is cut out from the parent body the mesenterial organs are extruded as an irregular mass of tissue. The very crude and indefinite operation of removing this mass by a transverse cut as indicated in Fig. 2 must accomplish the same general result as an operation along the broken lines in Fig. 31 would accomplish, provided such an operation could be performed, when the animal was in the distended condition. The two lines a and b, Fig. 31, serve to indicate roughly the results of the removal of less or more of the mesenterial organs, as this result would appear if the piece could regain a fully distended condition. It is evident from the figure, and examination of the pieces also shows very clearly, that the mesenteries are most completely removed in the terminal regions of the piece. Frequently the mesenteries are practically wholly removed at some regions of the circumference near the cut end, more frequently the oral end, since extrusion is usually greater In such cases nothing but the body-wall remains. But the results of the operation are never uniform about the whole circumference, at least not in the cases under my observation, and it is not likely that they can be in any given case. At some points

of the circumference the mesenteries are more completely removed, at others less completely; sometimes certain mesenteries are only slightly extruded, others almost entirely, so that the part of any given mesentery which is removed will differ greatly and cannot be controlled. But the important point is that those regions where the removal of the mesenteries is most complete near the cut end are apparently the regions where the greatest degree of change in the body-wall and the most active growth after closure occur. In other words, the regions where the greatest change results from the operation are the regions which show greatest evidence of that change in their later behavior.



F1G. 31

My observations of these pieces lead me to believe that, in at least many cases, those regions of the circumference where the mesenteries were most completely removed are the regions where groups of tentacles are most likely to appear. It is difficult to attain certainty on this point; after closure regions of greater transparency and more active growth can often be distinguished at different points of the circumference along the line, of union and examination of these regions shows either no distinct continuations in them of the mesenteries which, farther away from the line of union are visible through the body-wall, or else very delicate, evidently newly formed mesenteries which may connect with the old mesenteries.

It is to be expected that regions in which the original organization and correlation of parts have been more completely eliminated will present more favorable conditions for the establishment of a

new organization than others, provided they still remain capable of reacting to the factors which determine the new result. It seems probable then, not only from observation, but on general grounds as well, that the more complete removal of the old mesenteries from particular regions about the circumference of the oral end of a piece which forms a ring may constitute an important factor in determining the localization of tentacle groups at those regions. That this is the only factor involved, I should certainly not venture to assert at present. Evidently the union of oral with aboral end not only decreases polarity but also decreases or eliminates the correlations about the circumference so that different regions of the circumference react independently, at least in considerable degree. Whether a difference in growth activity is sufficient to accomplish this cannot be determined at present. From my previous experiments on actinians it is evident that different parts of the circumference are not normally very closely correlated, for each part performs its characteristic reaction according to the conditions present at that point and apparently without reference to other parts. The results of the lateral incisions which have been described both for Harenactis and Cerianthus indicate this very clearly. This being the case I am inclined to believe that the establishment of a region of more rapid growth and less differentiation at a certain point of the circumference might be sufficient to determine reaction of that region independently of other parts about the circumference.

If these suggestions are correct they afford some basis for understanding how it is that a number of isolated groups of tentacles may appear about the circumference of these rings. Perhaps other internal factors of which we know nothing are involved, but it is evident that such factors if present act in very indefinite and irregular manner, since restitution in rings varies from mere closure without the formation of tentacles at all to the formation of anywhere from one to six—the maximum number in my experiments, though doubtless not the maximum number possible—groups of tentacles in which the number and arrangement of the tentacles may also vary greatly. In short the determining factor or group of factors in the localization is very evidently what we

may call a chance factor and acts apparently without reference to anything in the original organization.

The other factors undoubtedly play a part, though incidentally, in determining where along the line of union tentacle groups shall appear and where they shall persist. The first of these factors is the distension of the enteric cavity with water. Water can enter these rings only through the body wall, of course, but like œsophageal pieces, they always become more or less distended after closure. Very frequently, however, certain regions become much more distended than others and in some cases distension appears to be confined almost wholly to certain regions. These local differences in distension are most marked in cases where a considerable portion of the mesenterial organs remains in the enteron and are undoubtedly due to the fact that some intermesenterial chambers are filled with the parts of these organs remaining and so do not share in the distension of other parts. My previous work on actinians has shown that a certain degree of distension is a necessary condition for the appearance and persistence of tentacles in restitution and this is true also for Harenactis. It may be expected, therefore, that tentacles will be more likely to develop in the regions of greater distension than in others. But this factor cannot determine the localization of the tentacles in radially symmetrical groups, and as a matter of fact such localization is most conspicuous in those cases where most of the mesenterial organs are removed (e. g., Fig. 29). It is very evidently only an incidental factor and one which affects rather the development and persistence of the groups than their localization.

In some cases the movements and contractions of the ring after closure bring about a change in position of the contained mesenterial organs and so a change in distension in certain regions. In some cases of this kind I have seen tentacle groups which had developed undergo reduction and atrophy because the opening into the enteric cavity of the ring was plugged with the mesenterial organs. Sometimes parts of the mesenterial filaments enter and fill the cavities of single tentacles and these tentacles undergo atrophy. The effect of distension or its absence is evident in these rings as elsewhere in regulation, but it certainly does not account

for the localization of definite tentacle groups about the circumference of the ring except as I have indicated above.

The other incidental factor is the presence of a layer of tenacious slime over the surface of the body. Harenactis, like Cerianthus, secretes this slime in greater or less amount according to condi-In the rings it is particularly annoying because it frequently encloses all parts of the ring. In my experiments I removed it as often as possible but it was usually secreted anew. Cylindrical pieces readily creep out of it but the rings are incapable of this. Very frequently this slime prevents the distension with water of tentacles which have formed, and if this condition is allowed to continue these tentacles may undergo complete atrophy. The atrophy of all but one tentacle of the group c in Figs. 24 and 25 and of the single tentacle at d in Fig. 24 (Cf. Fig. 25) was due to this slime. The single tentacle which persisted in the group c protruded through an opening. It seems probable therefore that this slime may in some cases prevent the appearance of tentacle groups by mere mechanical pressure. In a number of cases where the rings were left for a week or more without removal of the slime they became completely enclosed in it and no tentacle groups appeared. But while this factor like distension may prevent the appearance of tentacles or groups or may bring about atrophy of those already developed it is only an incidental factor, for the tentacles appeared in groups in cases where the rings were kept practically free of slime.

### 3 The Establishment of the New Polarity and Symmetry

Our conception of the nature of the processes involved in the establishment of a new polarity will of course depend on our conception of the nature of polarity. If we regard it with Driesch as a directive property or characteristic inherent in each ultimate particle, element, molecule, or whatever we choose to call it, of protoplasm, we must conceive a change in polarity as involving a coördinated change in direction of the axes of these particles. If we adopt Morgan's hypothesis that it is the expression of an

axial gradation of materials then changes in polarity consist in changes in this gradation. But neither of these hypotheses gives us anything more than a formal statement of the fact of change of polarity. Moreover, as I attempted to show in the preceding paper (Child '09b) neither of these hypotheses accounts for all the phenomena of polarity, though I believe that Morgan's hypothesis is much more nearly correct, so far as it goes, than Driesch's, for there is no satisfactory evidence in favor of a directive organization, while there is abundant evidence to show that specification or differentiation along the axis exists.

Correlation as well as constitution is an essential factor in organic polarity; in fact under certain conditions differences in correlation may determine differences in constitution, i. e., the constitution of a given region may be altered in one direction or another according to the character of its correlations with other parts.

It can, I think, be shown that the phenomena of organic polarity are possible in a system or individual in which the only localized differences of any kind are differences in the rapidity or intensity or both of reaction along some axis, but these phenomena are possible only when correlations exist. In such a system one region will react more rapidly or more intensely to a given stimulus than others and the correlative effect of this reaction, must necessarily be the alteration of the reactions in other parts. Moreover, the processes in the region of greatest reaction rapidity or intensity must produce a greater effect upon other regions than the processes in these regions produce upon it. In other words it becomes the dominant region of the system physiologically, and in the following structural and functional complication which must occur in such a system it is in advance of other parts and may determine more or less completely their fate, according to the nature and degree of correlation. I am inclined to believe that the ovum is essentially such a system, that in its simplest form the only "polarity" present may be a difference in the rapidity (or intensity) of reaction along some axis. It is evident that in very many cases, if not in all, the region of greatest reaction rapidity at the beginning of development, i. e., the "animal pole" becomes the head region or in more

general terms the dominant region of later stages. Doubtless many complicating factors exist in different cases, but they do not alter the fact that polarity reduced to its lowest terms may consist merely in a quantitative difference in reaction or in certain reactions along some axis.

If these suggestions are correct, it is evident that a polarity of some kind will arise wherever such differences in reaction or in reactive capacity are established along an axis. If such a region remains in correlation with the original whole so that the nature of its reactions is determined by its correlations, the result will be a polarity or symmetry of parts or organs. If, on the other hand, the correlations with other parts of the original whole are reduced or eliminated and if the cells involved in the new relations are totipotent, i. e., possess the same capacities or "potences" as the ovum, then a new system or individual must of necessity arise if life continues, and its principal axis must coincide in direction with the differences in reaction rapidity which constituted the starting point.

This very brief consideration of the problem is perhaps insufficient to show clearly the basis for my conclusions, but it must serve for the present. I hope to present a more complete statement of my position and the grounds for it at another time.

Returning now to the problem of the establishment of the new polarities about the circumference of the rings, certain points suggest themselves on the basis of what has been said. In the first place, if regions of more rapid growth are established at certain points in consequence of greater injury to the old system, as suggested above, a gradation in physiological activity between the areas of most rapid or most intense reaction and the less active regions about them must exist. I believe that this gradation represents the first step in the establishment of the new polarity. If the original correlations between the various parts still persisted these regions might undergo further development as parts of a whole, but we have seen above that union of the oral with the aboral end has altered these correlations, at least quantitatively, i. e., the original polarity has been decreased or almost eliminated. Consequently the different regions grow independently of each other, at least

in large measure. As each region grows it bulges out more and more from the level of the body-wall, forming a small, usually rounded elevation on which the tentacles later appear. It seems probable from my point of view that with the formation of this slight elevation the new polarity is established both as regards direction and position of the two poles. This establishment of the new polarity does not consist in any special "formative" process, but is merely the necessary consequence of the localization of the region of growth and of the growth occurring there. In fact it seems to me inconceivable that some sort of polarity should not arise in every region of growth which is definitely directed either by internal or external conditions. constitution of all parts involved is originally the same, the terminal region for example must become different from a middle region because the correlations cannot possibly be identical in these two regions and because they are differently related to the external world. In short, any organic system in which either the correlations between parts or the relations between the system and the external world differ characteristically in different regions possesses a polar specification in some degree.

In Harenactis as in most elongated forms the oral region is in general the initiating or dominant region in reaction, i. e., it reacts more rapidly than other regions in the presence of many, probably of most stimuli. So far as this is the case this region must play an important rôle in determining the development of other correlated parts, since the fact that it reacts to a given stimulus before they do usually or always changes the character of the reaction in them, according to their correlation with the dominant region.

In the regions of localized growth on the rings the central part, or after elevations have been formed, the terminal portions of these are undoubtedly the physiologically dominant parts, and as such develop oral structures. These regions of growth are then briefly more or less completely radially symmetrical regions in which polarity arises in consequence of their constitution and their correlations with adjoining parts. Such a system composed of Harenactis protoplasm being given, it seems probable that structural localization and differentiation must occur in greater or less degree,

and that, because of the character of the material, it must approach more or less closely to the usual course of development, according to the conditions.

In the rings various factors prevent the formation of complete animals and, as has been shown, these factors operate in different degree in different cases. The number of tentacles in the group is undoubtedly determined by the number of mesenteries. In cases where no new mesenteries are formed (e. g., Figs. 8, 10, 17) the number of tentacles is determined simply by the number of mesenteries involved in the region of localized growth (Figs. 18 to 20.) In cases such as No. IX (Fig. 29) where new mesenteries are undoubtedly formed, the number of mesenteries and tentacles is undoubtedly determined by the distance factor in correlation, i. e., by the distance within which the formation of one mesentery or a pair inhibits the formation of another.

#### 4 Localization of the Groups in the Oral-Aboral Direction

In the above description of the various cases they were divided into two series, those in which the groups formed on the line of union with some of the tentacles arising from the original aboral end of the body-wall and from the line of union itself (Cases I to V, Figs. 6 to 20), and those in which they were situated wholly on the oral side of the line of union (Cases VI to IX, Figs. 21 to 30).

As regards the conditions determining these two different localizations, my records indicate that the pieces in the second series were those from which the mesenteries had been more completely removed, at least near the oral end, than in the pieces which form the first series. In most of the pieces in the second series the m senteries were extruded and removed more than once, while in the first series only one extrusion and removal occurred in most cases. Referring to Fig. 31 in which the result of the removal of extruded mesenteries is indicated as it would appear if the piece could resume its original condition of distension, the condition in the first series is something like that represented by the removal of parts above the line a, while that in the second series is represented by removal of parts above the line b. Mesenteries were extruded

and removed from the aboral end in some cases in both series (c, Fig. 31), but with the usual method of experiment extrusion occurs chiefly from the oral end. Fig. 31 is of course purely diagrammatic and the different lines serve merely to indicate the effect of removal of more or less.

It is evident, however, that the more complete the extrusion and removal at the oral end the greater the distance over which the mesenteries are almost wholly removed (compare the lines a and b, Fig. 31). At present I am inclined to believe that these differences are factors in the oral-aboral localization of the tentacle groups. Apparently in those cases where the degree of injury at the two ends is not very different (e. g., after removal along a and c, Fig. 31) the tentacle groups form on the line of union between oral and aboral ends, while in those cases where the mesenteries are much more completely cut away from the body-wall at the oral end (e.g., by a second operation at b, Fig. 31, the groups are localized on the oral side of the line of union. In the first series of cases oral and aboral ends are involved in much the same degree in the formation of the new tissue at the line of union, while in the second loss of the old differentiation and growth occur to a larger extent on the oral side of the line of union. Since the tentacle groups are localized in the region of most active growth, they appear in the first series at the line of union and in the second on the oral side of it.

This is the only factor, if it is actually a factor, of importance for the oral-aboral localization of the tentacle groups, which is apparent from my observations. Whether this is the chief factor, or whether other factors are concerned, must remain for the future to determine. The only fact that might indicate that other factors play a part in determining this localization is that no cases have been observed in which some of the tentacle groups were on the line of union and some wholly oral to it: all the groups on each ring are apparently in the one place or the other. If the character of the operation is the chief factor the possibility of the appearance of both localizations in a single ring must be admitted, though it need not be expected in any given case. Further experiments are necessary to determine this point.

But whatever the factors involved the facts are of interest since they show a difference in localization, undoubtedly the result in some way of the experimental conditions, of the regulatory processes with respect to the wound. While such differences of localization are of frequent occurrence in plants and can often be controlled experimentally, they are much less common in animals.

#### 5 Correlations in the Formation of the Tentacle Groups

While correlations are necessarily essential factors in the formation of such systems as the tentacle groups, their effect is most conspicuous in certain particular features of these experiments, viz: in the formation of axially heteromorphic and radially heteromorphic tentacles. In the first series, where the tentacle groups form on the line of union the symmetry of the groups is more or less perfectly completed by the formation of tentacles on the aboral side of the line of union opposite those on the oral side (Nos. I to V, Figs. 6 to 20). In pieces where the oral and aboral ends close without uniting with each other tentacles never appear on the aboral ends, so far as my observations go, except in the œsophageal region, as already described. In all of the rings described, however, not only the aboral but the oral ends as well are from the subæsophageal region, yet in all of the first series (Figs. 6 to 20) tentacles appear on what was originally the aboral end of the piece. The only sufficient reason for the appearance of axially heteromorphic tentacles in the rings and not in other pieces involving the same region of the body is, in my opinion, the union of the aboral with the oral end in the rings. Apparently this union alters the character of the aboral end, at least quantitatively, so that it becomes capable of producing tentacles. Cases I and III are particularly good illustrations of the influence of the oral upon the aboral end. In both of these cases (Case I, Figs. 6 and 8; Case III, Figs. 11 and 12) the tentacles appear first on the oral side of the line of union, i. e., on the oral end, and later on the aboral side: Case II (Fig. 10) is similar to the other two but the early stages are not figured. Moreover, in these cases tentacles never appear at any point on the aboral end except opposite those

regions where tentacles have already appeared on the oral end: this is evident from Figs. 6 and 8, and 11 and 12. The only possible interpretation of these cases seems to me to be that the aboral end has become more oral physiologically in consequence of its union with the oral end or, in other words, the original polarity has been largely eliminated because the correlations have been altered. The other two cases of the first series differ from these only in the simultaneous appearance in most cases of the tentacles on both sides of the line of union (Figs. 15, 16 and 17 to 20).

In the second series, in which the tentacles appear wholly on the oral side of the line of union, that part of the group which lies nearest the line of union often appears later than the other parts and remains of smaller size. Such a case is shown in Fig. 23 which represents group b of Fig. 22 and the other groups of this ring are similar; in Fig. 24, groups a and c, two tentacles on the side nearest the line of union appear later and are smaller than the others, and in group b the fourth tentacle which lies on the side toward the line of union does not appear at all until after the stage shown in Fig. 24. Fig. 25, group b, and Fig. 27 show this group as it appears after the fourth tentacle has developed.

In these cases the part which lies nearest the original oral end of the piece develops tentacles more slowly than other parts. Apparently in these cases the development of these tentacles is delayed either because union of the oral with the aboral end affects the oral end, as it has been shown above to affect the aboral end, but in the reverse direction, or else the reversal of symmetry which is involved in the formation of these tentacles requires a certain amount of time, or both of these factors may be involved. inclined at present to believe that the effect of the aboral upon the oral end may be the more important in determining the rapidity of development of the tentacles in question. Since the aboral end, which otherwise never produces tentacles, becomes capable of tentacle-production when united with the oral end in these rings, it is not at all improbable that the physiological condition of the oral end is altered by its union with the aboral end, but in the reverse direction. If this is the case, the union of the two ends decreases the differences between them, i. e., the polarity. It is

certain that the ability of the oral end to form tentacles at all is much less in the rings than in other pieces; the time between the operation and the first appearance of tentacles is usually more than twice as long in the rings as in other pieces.

But, on the other hand, in many of the tentacle groups the tentacles all appear at the same time and are of equal length whether the groups are situated on the line of union (Figs. 15 and 16, 18 and 20) or wholly on the oral side of it (Figs. 28, 29 and 30). In general such groups are the most perfectly radially symmetrical, while those in which there is the greatest difference between tentacles on the oral and aboral sides are usually irregular (Figs. 8 and 10). In the radially symmetrical groups with equal tentacles the old polarity has simply been more completely obliterated than in the others before the formation of the tentacles, so that no appreciable traces of it remain in the region of union after the establishment of the new polarity.

These tentacle groups, then, show us various stages in the process of the obliteration of the old and the establishment of the new polarity. In the early stages of some rings, e. g., Figs. 6 and 11, the original polarity is still present in something like its original condition, for tentacles appear only on the oral side of the cut surface: later in these same pieces (Figs. 8 and 12) aboral tentacles develop. In many other cases the difference in size and time of appearance between the different tentacles is not as great, and they often become almost or quite equal in size in the later stages. And finally we come to such cases as No. V (Figs. 17 and 20) in which scarcely a trace of the original polarity is apparent in the tentacle groups, and No. IX (Fig. 29), where the original polarity and its effects are not at all visible in the groups: the only feature in such cases which can possibly be referred to the original polarity is the localization of the tentacle groups on the oral side of the line of union, although, as was shown above, this localization probably occurs only under certain experimental conditions.

I believe that the union of the two ends with each other and the physiological effect of this union upon each part are the chief factors in obliterating or decreasing the original polarity in this region and so producing conditions favorable for the establish-

ment of a new polarity. Analysis of the regulatory processes in these rings seems at present to afford strong evidence in favor of this view.

## 6 Origin and Structure of Oral-Aboral Tentacles

The frequent occurrence of oral-aboral tentacles along the line of union in the rings has been noted in the descriptive section of the paper. They occur in most of the groups which involve both sides of the line of union, and may be either simple like other tentacles or forked. In the figures of the first series of rings (Figs. 6 to 20) simple oral-aboral tentacles appear in the following cases: in the left-hand tentacle group in Fig. 10 the tentacle at each end of the group is oral-aboral, though apparently belonging more to the oral side than to the aboral; in groups b c, and d of Fig. 12 the two lateral tentacles are oral-aboral in each case, and in group a the two tentacles of medium length. In the case shown in Fig. 15 more oral-aboral tentacles than oral are present, for the two lateral tentacles in each group are oral-aboral; finally oral-aboral tentacles are present in each of the groups in Fig. 17, as shown by the detail figures 18 to 20. In Fig. 18 tentacles 1 and 4 are oralaboral, in Fig. 19, 1 and 3, and in Fig. 20, 1 and 3. In these three groups all of the oral-aboral tentacles except No. 4, Fig. 18, arise largely or wholly on one side or the other of the line of union, instead of upon it, as in most cases (Figs. 10, 15, 16). Nevertheless a valid reason for regarding them as oral-aboral tentacles is to be found in the fact that each arises over an intermesenterial chamber bounded by what was originally the oral end of one mesentery and the aboral end of another.

Forked or branched oral-aboral tentacles occur in Fig. 8 (a and b) and in the right-hand group in Fig. 10.

The appearance of these oral-aboral tentacles constitutes a demonstration of the fact that the original polarity and radial symmetry are almost or quite obliterated and a new polarity and symmetry established. The formation of a tentacle like those of the normal animal in form and function, so far as can be determined, from tissue arising in part from the original oral end, in

part from the aboral end, would undoubtedly be impossible if the original constitution and correlations existed.

The occasional appearance of forked or branched oral-aboral tentacles in which the oral and aboral parts of the tentacle are separate for more or less of its length are of some interest as "abnormalities." But forked and branched tentacles are by no means confined to the line of union between oral and aboral ends in rings. They occur not infrequently in other regions in rings, in Fig. 18, for example, in an oral tentacle, in Fig. 21 a and in Fig. 29 a and f in groups which are wholly on the oral side of the line of union. Moreover, they are often seen in other cases of restitution.

Though their method of origin differs in different cases certain features are, I think, common to all, whether they are oral-aboral or in the usual position. The growth of tentacles in restitution occurs chiefly in the more proximal regions, the distal portions of the tentacle being the first to appear: in fact, there is considerable evidence, which I hope to present at another time, that in at least some actinians the tentacles are throughout life growing at the base and undergoing atrophy at the tip. It follows from the method of growth of the tentacles that the branched portion was formed before the single basal part, i. e., such tentacles began to form as two distinct tentacles but later united. In a number of cases this sequence of events has actually been observed, so that no doubt of its occurrence can exist.

The factors which determine the occurrence of fusion or union between the two parts which are at first distinct are, so far as I can determine, the following: first, the diameter of the first portions of the tentacle to appear, i. e., the distal portions, is usually less than that of later, more proximal portions, especially if the developing tentacle is growing rapidly; if two regions of tentacle-formation are localized near each other they may form separate tentacles at first, but as each increases in size they may meet and unite to form a single region, so that further growth is single, not double. A second factor producing essentially the same result is the division into two parts of a tentacle-forming region in its earlier stages. Such division often occurs in restitution in consequence of folds and wrinkles in the body-wall which result from the contraction

following the wound. In the closure of the oral end of a piece in the usual manner (Child, '09a) folds extending radially from the contracted cut end commonly occur and may pass through a region of tentacle-formation. In consequence of lack of distension, and perhaps also for other reasons, growth is retarded or inhibited in the fold, and in forms with delicate body-wall like Cerianthus æstruarii (Child, '08) complete atrophy and degeneration of the folded or wrinkled regions may occur. Sometimes these folds involve a portion of the body-wall so large that the formation of one or more tentacles is inhibited or delayed. Cases of this sort in Cerianthus solitarius and C. membranaceous were described in an earlier paper (Child, '04, pp. 281 to 284, Figs. 5 to 7). On the other hand, very slight folds may divide a tentacle-forming region into two parts: localized growth may occur on each side of the fold, but growth in the fold itself is retarded or inhibited, consequently two tentacles begin to develop where only one would have formed if the fold had not been present. But in all the actinians which I have used for experiment the increasing distension following closure and mouth-formation and the gradual redifferentiation of the body-wall near the cut end usually bring about sooner or later the disappearance of these folds. In the case of folds dividing a tentacle-forming region the disappearance of the fold is followed by fusion of the two growing tentacles and their further growth as a single tentacle. Cases of this kind can often be observed in pieces undergoing regulation: they are not essentially different from other cases in which experimental division of the distal part of a regenerating primordium determines the differentiation of two structures instead of one, e. g., the starfish arm and the amphibian leg.

In the case of forked or branched oral-aboral tentacles on the rings the first factor mentioned, i. e., the union of two tentacle-forming regions which are close together, in consequence of their increase in size, is probably more frequently concerned than the other. It is possible, however, that the cells immediately adjoining the cut surfaces, i. e., those cells which accomplish the union between oral and aboral ends in the rings react less readily, perhaps because of their exposure at the cut before union or for other

reasons, to the "tentacle-forming stimulus" than cells slightly more distant from the cut. If such is the case the localization of a tentacle-forming region on the line of union may be followed by more rapid growth on the two sides of the line of union than at the line of union itself. Under such conditions the line of union might produce the same result as a fold dividing the tentacle-primordium and a distinct small tentacle might appear on each side of it. Later fusion of the two tentacles might occur in consequence of correlative change in the constitution of the cells of the line of union.

But whatever the exact process in a particular case, all of these forked and branched tentacles are the result of fusion of localized regions of growth in consequence of the obliteration of regions of less rapid or inhibited growth between them. Such tentacles are then, properly speaking, not forked or branched but rather partially fused tentacles.

One tentacle with oral and aboral portions, found on the ring shown in Figs. 8 and 9, is of particular interest. Both of the tentacles indicated by the letter a in Fig. 8 are apparently oral tentacles, but each of them has what we may call an aboral branch at about the lower third of its length. The plane of section in Fig. 6 passes through one of these two tentacles, this being the one which chiefly concerns us at present—the tentacle appears in the left half of Fig. 9. This tentacle began its development as two distinct tentacles, one on each side of the line of union. The oral part which is the longer of the two distal branches appeared somewhat earlier than the aboral. Later, as the diameter of the two growing tentacles increased, fusion occurred, and since the oral part was the larger, the aboral part became what seems to be a shorter branch of it and, in consequence of continued growth of the basal fused region, came to occupy the position shown in Fig. 9. The line of union between oral and aboral end of the piece is represented in this tentacle by the region where the aboral and oral branches unite, i. e., the base of the fork. But the feature of particular interest in this case is the later formation of another aboral tentacle opposite the base of this branched tentacle. This tentacle appears in surface view on the upper left

side of Fig. 8 and in section in Fig. 9 (it is the short tentacle pointing toward the left on the left half of Fig. 9). Why this second aboral tentacle should have formed in this case alone it is impossible to determine, but the fact itself is of interest. This tentacle is formed from tissues which, in other cases have nothing to do with tentacle-formation; moreover, its relation to the line of union i. e., the original cut surface, is different from that of other tentacles. I suggested in an earlier section that the formation of tentacles on the aboral side of the line of union in these rings is in all cases the result of correlation with the tentacle-forming regions on the oral side and that suggestion applies to the present case as well as to others. Whether the correlation is direct or indirect and what its nature may be we cannot now determine. It seems probable, however, that it represents some approach to the establishment of a new symmetry, or, in other words, where conditions exist which determine the formation of one tentacle, they are probably favorable for the formation of others in a more or less definite arrange-This statement does not carry us far toward a solution of the problem, but the evidence which these rings afford in favor of correlation of some sort in tentacle-formation is at least of some value.

It is perhaps worth while to emphasize the fact that this second tentacle on the aboral side appears in a quite different relation to the previously existing wound from that in which tentacles usually appear. One point in Driesch's criticism (Driesch '05, p. 776) of my earlier work on Cerianthus was to the effect that I ascribed no localizing influence to the wound. While this criticism was not correct, the fact that the relation of tentacles to the region of the wound may differ under different conditions is nevertheless important since it constitutes positive evidence. And this fact is, I believe, established by the case described above.

#### III CONCLUSION AND SUMMARY

The phenomena of regulation in these rings are of considerable interest, not as "abnormalities" or "errors in regulation" but as reactions under conditions different from those usually present.

While we are far from a complete analysis of these reactions and the conditions upon which they depend, there is every reason to believe that they are as characteristic under certain conditions as the "normal" reaction is under others.

But whatever the particular factors involved, the rings show how readily polarity and symmetry may be altered or obliterated and new polarities and symmetries arise. Moreover, they afford an excellent illustration of the readiness with which an organic system breaks up into smaller systems when the old correlations between parts are obliterated. The union of parts into a whole seems to me to be essentially a matter of correlation: if this is the case, the whole must cease to exist, at least physiologically, when the correlations are eliminated or altered beyond a certain limit. But whether, under these conditions, new wholes shall or shall not be formed, will depend first on the capacity of potence of the material and second on the local conditions in various regions. If, for example, local conditions determine in totipotent material, the establishment of a new polarity in any region or regions a new whole or wholes may arise. Probably, as was suggested above in discussion of the establishment of the new polarities, polarity in its simplest form may arise in consequence of very simple or very slight local differences in living material. Whether such polarity becomes the polarity of a whole or of a part will depend likewise on the character of the material involved and the environmental conditions (internal correlations and sometimes external conditions) which are present.

In the rings it is evident that new polarity and symmetry are established, but in consequence of gradual collapse in all cases, growth does not continue sufficiently far to show whether the new systems would finally reach a condition of equilibrium similar to that of the old, or differing from it more or less widely. If a tentacle group with a mouth should appear on one of these rings it would probably be possible to keep the whole complex alive indefinitely.

Those parts of the tentacle groups which arise from tissue belonging to the original aboral end of the piece (Cases I to V, Figs. 8 to 20) are of course axial heteromorphoses; moreover, they are secondary heteromorphoses (Child '09b), since the elimination of the original polarity is the result of conditions which did not exist before isolation. But these cases are different from the usual form of axial heteromorphosis, forthey involve a change in the axes of symmetry as well as polarity. In the second series (Cases VI to IX, Figs. 21 to 29) where the groups of tentacles arise wholly on the oral side of the line of union there is no polar heteromorphosis like that in the first series, but "radial heteromorphosis" is present. But the question as to whether a given case is or is not heteromorphosis seems to me of minor importance: we have simply to recognize the fact that in all of these cases the original polarity and symmetry are more or less completely obliterated and that polarity and symmetry of the new systems are more or less completely determined by local conditions in the material. In fact, as was noted above, comparison of the different cases shows that in some the old polarity and symmetry evidently play a part in determining the arrangement within the group (e. g., Figs. 8 and 10), while in other cases they do not appear at all (Fig. 29), unless the position of the tentacle groups on the oral side of the line of union is a consequence of the original polarity, which may or may not be the case.

The restitutional phenomena in these rings are, in my opinion, cases of division of a system in consequence of decreased correlations between parts. Asexual reproduction can be induced experimentally not only by inducing growth but by decreasing the correlations between parts. It is possible, for example, as I have recently discovered, to induce fission experimentally in individuals of Planaria dorotocephala of any size above six or seven millimeters in length, even though they may have been decreasing in size from lack of food. The method usually employed is simply the removal of the head; frequently, especially in small individuals, it must be removed several times as it regenerates. The removal of the old head decreases the correlation—in this case nerve coordination, at least in large part-between the anterior and posterior regions, and under these conditions the development of the new zoöid in the posterior region is hastened, until sooner or later it becomes sufficiently independent to show independent motor reaction, i. e., to attach itself to the substratum independently of the anterior region, and then separation occurs. These experiments will be considered more fully clsewhere, but it seems to me worth while to point out that the tentacle groups on the rings are a kind of asexual multiplication, i. e., a division of the system which is determined primarily by changes in the correlations of parts, which decrease or obliterate the original polarity and symmetry.

Asexual reproduction is very frequently due, as I hope to show elsewhere, to a decrease in correlation or to escape of certain parts from correlation. In my consideration of the formation of aboral hydranths in pieces of Tubularia (Child '07) I touched upon this point and showed that the various phenomena could readily be accounted for on this basis. In the case of Harenactis asexual reproduction does not occur or occurs very rarely in nature, but under the peculiar conditions existing in the rings a certain kind of asexual reproduction does take place. While it does not produce individuals capable of long-continued existence, this feature is undoubtedly incidental, and it seems at least highly probable that the formation of a number of more or less independent systems, the tentacle groups, in the rings is physiologically very similar to many cases of asexual reproduction which occur in Moreover, as I suggested in connection with Tubularia (Child, '07a, b,) all cases of axial heteromorphosis, whether primary or secondary, undoubtedly belong in the same category.

The principal points of the paper may be summarized as follows:

- I Rings are formed from cylindrical pieces of the body of Harenactis by the union of the oral with the aboral end about the whole circumference. Their formation can be induced experimentally by removing a larger or smaller part of the mesenterial organs from the piece, and in the œsophageal region by removing the œsophagus. In general, the shorter a piece the more likely it is to form a ring in closing: when the length of the piece is less than the diameter a ring is usually formed or closure does not occur at all.
- 2 After closure the parts of the ring commonly undergo a revolution about a circular axis, so that the line of union between

oral and aboral ends lies at or near the equator on the outer surface, but changes from this position may occur.

- 3 In some cases regulation goes no further, but usually one or more groups of tentacles appear at or near the line of union. The tentacle groups consist of varying numbers of tentacles—in my experiments from one to twelve—and show various degrees of approach to a perfect radial symmetry, the one extreme being a series of tentacles on each side of the line of union, the other a perfectly radially symmetrical group.
- 4 The localization of the groups along the line of union is very irregular and is apparently not determined by any factor directly connected with the original organization. Different degrees of injury to different parts of the circumference probably constitute a factor in their localization.
- 5 In some cases the groups appear on the line of union and part of the tentacles arise from the original oral end of the piece, part from the aboral. Often oral-aboral tentacles appear, i. e., tentacles in the formation of which both oral and aboral ends take part. In other cases the whole group arises on the oral side of the line of union. Differences in the degree of injury of the two ends of the piece and perhaps also certain features of the original polarity, which persist, are probably factors in determining this localization.
- 6 The formation of the tentacle groups undoubtedly involves the establishment of a new polarity and in greater or less degree of a new radial symmetry. The original polarity disappears to a greater or less extent in consequence of the union of the oral with the aboral end. The establishment of the new polarities and symmetries is undoubtedly due to local factors in the regions where the groups appear.

These cases of regulation, like axial heteromorphoses, are to be regarded as a breaking up or division of the original system in consequence of decrease or elimination of the original correlations: they are in short a kind of asexual reproduction, the differentiation of new systems being the result of the localization of regions of growth at various points in the tissues of the piece.

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# AN ANALYSIS OF THE RATE OF REGENERATION THROUGHOUT THE REGENERATIVE PROCESS<sup>1</sup>

BY

#### MARION L. DURBIN

WITH SIX FIGURES

The experiments upon which the present paper is based were undertaken with the object of obtaining a comparison of the rates of regeneration throughout the regenerative process. zani,2 the first to study the rate of regeneration, makes this statement concerning the legs of the aquatic salamander: over, as natural legs take their greatest increase when they are still soft, and lengthen less when they begin to harden, the same thing happens in reproductions." "It is necessary to note the following periods, at least when we speak of salamanders already come to their growth, viz: the considerable time, which passes after the cutting off of the leg, before the reproduction begins; its slowness in the first period; its quick progress afterwards; and lastly its tardiness when the leg begins to harden." It is to be expected that the rate of regeneration of the tails of frog tadpoles will also show more or less regular change during the process. A study of this change should be of interest, (1) in comparison with the curve of ordinary growth, and (2) as a help in isolating the factors controlling the process of regeneration. These experiments dealt with tadpoles of Rana clamitans (Latreille) which were more than nine months old. The principal interest is confined to the increase in the length of the regenerating tail since an attempt to study its volume was unsuccessful. The rate

<sup>&</sup>lt;sup>1</sup> These experiments were performed in the Zoological Laboratory of Indiana University during the school year 1907-1908. I wish to thank Dr. Charles Zeleny for his careful directions and many helpful suggestions which have enabled me to do this work. I am also indebted to Mr. Max M. Ellis for the use of the data referred to on p. 411, Fig. 5. The present paper is listed as No. 105 of the contributions from the Zoological Laboratory of Indiana University.

<sup>&</sup>lt;sup>2</sup> Spallanzani (Lazaro) Abbé (1769). An Essay on Animal Reproductions.

at which the differentiation of the materials in the new tail takes place was also investigated. It was found that immediately following the removal of a part of the tail the rate of increase in length is slow, but rises rapidly to a maximum which it reaches on the sixth to tenth day. From this point there is a rapid decrease for about three to five days and then a progressively slower decrease until the process is complete. The curve expressing these rate changes is shown in Figs. 1, 2, and 4. It agrees in general features with the curve of the ordinary growth of rabbits and guinea-pigs as it is described by Minot.<sup>8</sup> These animals, soon after birth, but not immediately, attain a maximum rate of growth from which the decrease is rapid at first and gradual later. In connection with the factors controlling the process of regenerations the changes in rate show that the full force of the tendency to regenerate is attained within a short period after the operation, but that the retarding factors appear early and increase in intensity rapidly at first and then more slowly until they have completely overcome the positive growth tendencies.

#### MATERIAL

Tadpoles of Rana clamitans (Latrielle) were used for this work because: (1) They are abundant and easily obtained in large numbers. (2) They are capable of accommodating themselves to laboratory conditions, a fact shown by the low per cent of mortality in series maintained under observations for from one to two months. (3) They resist in a very satisfactory way the shock of an operation. (4) The tail offers a convenient appendage for operation and observations, as more than half its length can be removed without seriously injuring the animal; at the same time it has a high regenerative potential so long as the individual has not begun to grow its forelegs. (5) It is always easy to distinguish the regenerated part of the tail from the old part, that is the line

Minot, C. S. '08: Age, Growth and Death.

<sup>&</sup>lt;sup>4</sup> Kammerer, Paul, <sup>2</sup>05: Ueber die Abhängigkeit des Regenerationsvermögens des Amphibienlarven von Alter, Entwicklungsstadium und spezifischen Grösse. Archiv für Entwicklungsmechanik, Bd. 19, Heft 2.

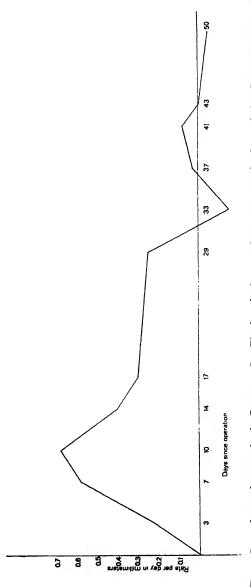
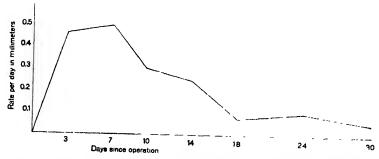


Fig. 1 Curve of rates of regeneration for Experiment I. The days elapsing since the operation are placed upon the base line. The rates at which the tail is increasing (or decreasing) in length are measured above or below the base line at the end of the corresponding periods.

upon which the operation was performed is always distinct. The difference between the regenerated and old parts of the tail lies in the facts that the regenerated part is unpigmented; that it is thinner in proportion than the old part, and that after operation the large blood-vessels near the cut turns blackish while those in the new tissue are colorless or reddish with their blood contents. The diameter of the notochord in the regenerated part is always distinctly less than in the old part, but as the old notochord is often pushed out a short distance (0.5–1 mm.) into the new tail the constriction on the notochord is not an infallible point from



- Fig. 2 Curve of rates of regeneration for Experiment II. For explanation, see Fig. 1.

which to measure the length of the regenerated tail. The measurements of length of the regenerated part of the tail were made along the notochord, from a point on the line of operation over the notochord to the tip of tail.

## **METHOD**

The technique employed has been very similar in the four experiments performed. Tadpoles collected from a pond were put into individual glass berry dishes containing about 150 cc. of tap water and some Spirogyra. There were two series of animals used in each experiment, except III, an uninjured or control series and an operated series. All of the animals used in an experiment were as much alike as possible. The two series were made to correspond, individual for individual, in regard to com-

plete body length and tail length. It was assumed that tadpoles of a given species living in the same pond and varying less than five millimeters in length are of approximately the same age. Operations consisted in cutting off a selected amount of the tadpoles' tails, the amount depending upon the size of the animals used in the series. All operations were performed with a razor while the tadpole lay on a block of paraffine; special care was taken that the line of operation be at right angles to the notochord, as occasional individuals with obliquely severed tails have shown an inhibition in rate during the later stages of regeneration. The length of the regenerating part of the tail was measured every third or fourth day at first; but after the greater changes in rate were past and additions to the new tail were being made very slowly, measurements were made less frequently in order that the differences between the last amounts recorded might be above the limit of error in the measurements.5

Each tadpole was kept in a glass dish with 150 cc. of water and more Spirogyra than the animal would eat before a new supply was given to it. Water and Spirogyra were changed every three or four days throughout the experiments. The tadpoles of experiment IV were fed upon an alga of the genus, Oedogonium. A change of food was made in Experiment III with marked results. Spirogyra was chosen as a laboratory food because the tadpoles would subsist upon it in a healthy condition for a long time, and because it was a help to the conditions of the water instead of a source of contamination.

The temperature under which these experiments were performed was not constant, but as it varied very little from day to day and the greatest variation was from day to night and took place in the cycle of every twenty-four hours, this factor is probably not a source of much inaccuracy. The irregularity in the gradual

<sup>&</sup>lt;sup>8</sup> Measurements of body length and tail length were made with dividers and a millimeter rule while the animal lay upon a pane of glass over a tilted mirror. Body length is the distance from the tip of the snout to the tip of the tail, tail length is the distance from the angle the tail makes with the body at the level of the notochord to the tip of the tail. The first two measurements of the regenerated part of the tail were made with an ocular micrometer and are accurate to 0.04 mm. The succeeding measurements were made with the dividers and are accurate within 0.30 mm.

decrease of the rate late in experiment II shows the effect of a prolonged period of reduced temperature.

Error from individual variation was eliminated by removing from the series all strikingly abnormal individuals and by basing the results of the experiment upon an average of a large series of individuals. (See Table II.)

#### DATA

Experiment I. March 8, 1908, twenty individuals 40-43 mm. in length were operated upon. The average amount of tail removed was 15.1 mm., the extremes of the operation were 17 mm. in one case and 14 mm. in two cases. The set was composed of nineteen individuals in an operated series and twelve individuals in a control series. The average body length of the operated series was 41.1 mm., of the control series 41.2 mm. During the last of this experiment the animals were subjected to two irregularities of treatment. They were left without food

TABLE I

	-			-						-
-	MARCH							APRIL		
Dates of measurements	8 11	15	18	22	25	6	10	14	18	28
in mm		2.95	5.00	6.60	7.60	9.68	9.20	9.30	9.60	9.40
measurements Gain during periods in	3	4	3	4	3	12	4	4	4	10
Gain during periods in mm	0.68	2.27	2.05	1.60	1.00	2.08	0.52	4 0.10	0.30	0.20
Rate during periods	.230	.570	.680	.400	.3∞	.170	.130	.025	.075	.020
Measurements of control series in millimeters	41.20	; !	40.17			į		40.10		38.9

Data for Experiment I. The first horizontal row of figures gives the dates upon which each measurement was made; below each date is the average amount of tail in millimeters regenerated by the operated series at that time. The third row of figures gives the number of days that have elapsed since the last measurement. The fourth row gives the gain in length during these periods. The fifth row contains the rate in millimeters per day, at which the tail grew during each period. The measurements of body length are the averages for the control series, and are given in the columns headed by the dates upon which the measurement was made.

for four days at one time and allowed to live in foul water for four days at a later period. The set was discontinued April 28, fifty days after operation. Table I gives the averages of the results of the measurements made on this set.

Experiment II. Tadpoles used in Experiment II were 33-35 mm. long; the average for the set was 34.6 mm. The operated series was composed of twenty-three individuals, and the control series of twelve. The average amount of tail removed by the operation performed, April 27, was 11.5 mm. The extremes of operation were 9.7 mm. in one and 12.6 mm. in two cases. These series were discontinued on May 27, thirty days after the operation. Table II gives the complete data for the set and shows that individual variation is not sufficiently great to affect the validity of the averages. Table III gives the averages of the results obtained in Experiment II.

Table II gives the complete individual data for Experiment II. The first column gives the body lengths of the individual tadpoles. The first twelve belong to the control series. The second column gives the tail lengths of the tadpoles. The third, the amount, in millimeters, that was removed by operation on April 27. The next seven columns give the amounts regenerated upon the dates indicated at the heads of the columns. The remaining four columns give measurements of the tail and body length upon the dates indicated. The average for the series is given at the base of each column. Separate averages for the body lengths and tail lengths of each series and an average body length for the entire set is given.

Experiment III was made upon ten tadpoles 41.4-44.9 mm. long, the average for the series being 43.7 mm. All ten individuals were operated upon October 1, an average amount of 14.8 mm. was removed, the extremes of operation being 14 and 16 millimeters. The experiment is of interest in this paper, because, (1) measurements were made more frequently immediately after the operation than in the other experiments; and (2) a change of food later in the course of development of the new tails produced a change in the rate. The series was discontinued November 3d, but, as two individuals were killed for the histological

TABLE 11
Complete Data for Experiment II

:										-		<del>-</del>	
BODY LENGTH	TAIL LENGTH	APRIL 27 AMOUNT REMOVED	APRIL 30 AMOUNT RECENERATED	MAY 4 AMOUNT REGENERATED	MAY 7 AMOUNT REGENERATED	MAY II AMOUNT REGENERATED	MAY 15 AMOUNT REGENERATED	MAY 21 AMOUNT REGENERATED	MAY 27 AMOUNT REGENERATED	MAY 21 BODY LENGTH	MAY 21 TAIL LENGTH	MAY 27 BODY LENGTH	MAY 27 TAIL LENGTH
34 9 33 0 34 0 33 0 34 7 34 1 34 7 34 1 34 7 34 2 35 0 35 0 35 0 34 3 34 0 33 2 35 0 34 3 34 0 33 0 34 0 35 0 36 0 37 0 38 0	20.0 18.8 21.5 21.7 21.4 21.0 22.0 21.3 21.0 21.0 20.6 21.5 22.0 20.5 22.0 20.0 22.0 20.5 21.7 21.7 21.0 20.5 21.7 21.7 21.0 20.5 22.0 22.0 20.0 21.1 22.4 21.7 21.0 20.5 22.0 22.0 22.0 22.0 22.0 21.1 21.7 21.0 20.9 20.5 22.0 21.1 21.0	11.4 11.0 10.9 11.0 11.8 11.8 11.6 11.9 12.0 12.6 11.4 12.0 11.4 12.0 11.5	2.0 1.5 0.9 1.8 2.0 0.5 0.9 1.1 2.0 1.7 1.9 1.0 1.5 1.3 1.7 1.1 1.8 1.8	3.8 3.1 2.8 4.1 3.6 3.9 2.9 4.0 3.1 3.3 3.9 4.0 3.1 3.3 3.9 4.0 3.1 3.2 3.5 3.5	4.7 4.0 3.6 6.1 4.9 4.3 4.0 5.3 4.1 3.6 4.5 5.0 3.7 4.2 4.6 3.9 5.0 4.8 4.1 4.2 4.6	5.3 5.0 4.2 6.2 5.6 5.2 4.7 5.1 5.5 5.5 5.0 5.2 5.4 4.1 5.5 5.0 5.3 5.3	6.4 5-3 died 6-5 5.8 5.6 4.8 6-5 5.0 5.0 5.9 5.4 abnormal 6.0 5.8 6.1 5.0 6.0 5.2	6 9 5.5 6.1 6.0 6.0 5.6 7.0 5.4 5.0 6.1 5.9 6.0 5.8 abnormal 6.9 5.5 6.1 5.0 6.4 5.0	7.0 6.6 5.4 6.0 6.6 died 7.5 5.9 5.5 5 6 7.4 6.1 6.5 abnormal 7.0 6.2 6.3 6.0 7.4 5.0	31.0 32.8 32.5 31.6 32.1 31.0	20. 21. 22. 20. 21.5 21. 22. 20. 20.	32.0	21.0
34.2	21.2	11.5	1.4	3.4	4.3	5.2	5.5	6.2	6.5		age a	moun d	ts,

TABLE III

Data for Experiment II. Same explanation as for Table I

Dates of measurement	27	30	4	7	11	15	21	27
Amounts of regeneration in mm		1.4	3.4	4.3	5.2	5.5	6.2	6.5
Days in period between measurements		3	4	3	4	. 4	6	6
Gain during each period		1.4	0.2	09	0 9	0.3	0.7	0.3
Rate during each period		0.46	0.50	0.30	0.25	0.07	0 12	0 05

material in the regenerating tail on the fifth day after operation and two others four days later, individual variation probably has a large influence upon the average results after the ninth day from the time of the operation. The average results for this series are given in Table IV.

TABLE IV

Data for Experiment III. Same explanation as for Table I

	; [			o	стовен				
			-			-		-	
Dates of measurements	1	3 0.23	5	7	, 9	12	14	17	20
mm. of regeneration		0.23	1.31	3.34	5.35	7.55	9.07	9 17	9 60
Days in periods between measure-	•	i		1		; }			
ments	•	2	2	2	2	3	2	3	3
mm. gains during each period	1	0.23	1 08	2.03	2.01	2.20	1.52	0.10	0 43
mm. per day during each period	1	0.12	0.54	1.02	1.05	0.73	0.76	0.03	0.14
	1_	i	-	l	i .	ı.	·		1

Experiment IV was planned for a study of the histology of regenerations in relation to the changes in the rate. On October 17, 1908, the series containing thirty-five individuals was operated upon. The average amount removed was 14.8 mm., the extremes of operation were 14 mm. and 15.9 mm. A control series of five individuals was maintained. The animals were 45-50 mm. long, the complete set having an average length of 49.1 mm. Ten measurements were made in the thirty days following the operation and three individuals selected at random from the operated series were killed each time a measurement was made. Thus the last few measurements are influenced by the individual varia-

tion of the few animals left to represent the series. However, it is not probable that this factor entered into the results until after the sixth or seventh measurement was made. Table V gives the results for this experiment.

TABLE V

Data for Experiment IV. Same explanation as for Table I.

							-			
			остовкі	<b>.</b>			NOVEMBER			
Dates of measure-	1		ļ	l	ı	!	;			
ments	20	22	24	27	29	31	3	7	111	15
regeneration	0.86	2.15	3.66	5.20	5.95	6.38	7.10	7.60	8.20	8.40
Days in period be- tween measure-		; 						!		
ments	3	2	2	3	2	2	3	4	4	4
each period	0.86	1.29	1.51	1.54	0.75	0.43	0.62	0.50	0.60	0.20
each period	0.290	0.645	0.755	0.510	0.370	0.215	0.206	0.125	0.150	0.050
Measurements of	i								1	
ınm 47.10				46.04					45.10	44.50
					'			-		

The structure of the regenerating tail was studied in each of the stages represented by the three tadpoles that were killed every time a measurement was made. As the earliest stage at which material was killed came three days after the operation very little can be said with certainty concerning the origin of the cells in the regenerated tail. Barfurth has carefully described the beginning of regeneration in the tail of amphibian larvæ and his description of the process agrees very well with what can be made out from the present preparations. The points bearing on this study are: (1) The cells of the ectoderm are proliferated from the innermost layer of the old ectoderm. (2) The cells from which the core of the new notochord arises come from cells that form a layer, just inside of the wall of the old noto-

<sup>&</sup>lt;sup>6</sup> Barfurth, D.: Die Erscheinungen der Regeneration bei Wirbeltierembryonen. Handbuch der Entwickelungslehre der Wirbeltiere. Herausgegeben von O. Hertwig. Jena. 1903.

chord. The wall of the new notochord like that of the old is formed by connective tissue which is deposited in concentric layers. (3) The fibrous connective tissue that fills the fins of the new tail comes from the descendents of fiber forming cells present in the old tail—i. e., the fiber forming cells of the old connective tissue multiply, giving rise to masses of undifferentiated cells which are seen in the early stages of regeneration. Later these cells differentiate into the connective tissue of the new tail. The connective tissue in the fin and around the notochord seem to be of similar origin. (4) The muscles appear relatively late in the development of the new tail. The partially undifferentiated cells, sarcoblasts, that develop into new muscle fibers are described by Barfurth as coming from the muscle fibers of the old tail that are near the level of injury. (5) The new nerve cord is an outgrowth of the old. It regenerates more rapidly than the notochord or muscles.

It is interesting to note that in all the tails sectioned the old notochord projects a short distance into the new tissue of the regenerating tail. The changes due to differentiation in each state of development represented by a measurement of the regenerations of the operated series in Experiment IV, are shown in the following chart. The regions referred to in the chart are represented diagrammatically in Fig. 6.

#### DISCUSSION OF DATA

Before entering upon a discussion of the data presented by the operated series of these experiments it is to be observed that the tadpoles in the control series did not grow during the experiment. Table II includes the record of the control series during the course of Experiment II. Since the animals were not growing, any increase in length noticed in the operated series was due to the regeneration of the part of the tail removed by the operation.

The foregoing data show that in each series operated upon the rate of regeneration immediately after the operation is slow; it then increases rapidly until the maximum is reached, then decreases rapidly at first but gradually more and more slowly.

The graphic method probably gives the most satisfactory representation of these changes in rate. A curve of rate may be constructed using the abcissæ as the intervals since the operation and the ordinates as the rates of length regeneration per day. Minot expresses the rate of growth as per cent of increment per day; that is, the amount of increase each day is divided by the weight of the animal at the end of the day before. These curves of regeneration rates are not per cent of increment curves, because the

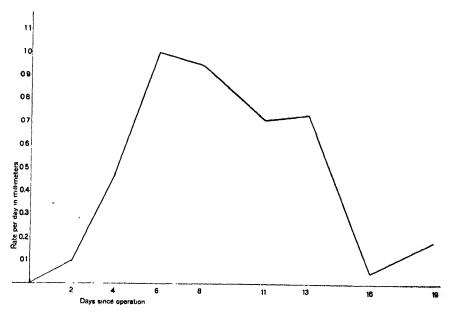


Fig. 3 Curve of rates of regeneration for Experiment III. For explanation, see Fig. 1.

amount of material added each day is so small in comparison with the length of the rest of the tadpole that the latter can be considered a constant.

The curve of rate of regeneration is made up of four distinct regions showing the rate to have undergone four distinct kinds of changes. The first is the initial region of low rate; it is most clearly exemplified in the curve of Experiment III, Fig. 3, in which the first measurement was made two days after the opera-

tion, when .23 mm. had been regenerated at the rate of 0.11 mm. per day. The measurement for the fourth day (1.31 mm.) shows the rate for the second two days to be 0.54 mm. per day. This interval of initial low rate probably extends over about two days. The first measurements in Experiments I, II and IV were not made until the third day when the tail had begun to grow faster. The rate for the first period in these cases is therefore a compound of the low rate of the first interval and the relatively higher rate apparent in the second region of the curve. The second region of the rate curve represents the interval of maxi-

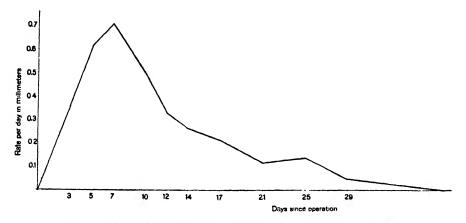


Fig. 4 Curve of rates of regeneration for Experiment IV. For explanation, see Fig. 1.

mum length adding activity. It ends when the rate has reached its maximum and has begun to decrease. The duration of this interval varies in different experiments; it is shortest in Experiment IV, where it extends over four or five days ,and longest in Experiment I in which it ends on the tenth day after the operation. The factors entering into the variation in the length of this interval and the rate at its maximum cannot be made out from the present data, but Ellis<sup>7</sup> has shown that they are in part at least dependent upon the amount removed. The third region

<sup>&</sup>lt;sup>7</sup> Ellis, M. M. <sup>2</sup>09: The Relation of the Amount of Tail Regenerated to the Amount Removed in Tadpoles of Rana clamitans. Jour. Ex. Zoöl. vol. vii, no. 3

of the curve or region of rapid decline represents a sudden decrease in the rate, whereby it is reduced to nearly half its maximum in three to five days. The fourth region is one of gradual decline representing the time of slow additions to length which terminates, in all probability, in the suspension of further regeneration of length. The third and fourth regions of the curve are not distinctly separate from each other; this is especially evident in the fourth experiment in which the transition between the two regions is more gradual than in the others because measurements were made more frequently.

It is also evident that tadpoles of this species do not regenerate as much tail as has been removed. There is, however, no reason to believe that this fact influences the curve of regeneration. In Experiment I a series maintained for fifty-one days had ceased to show increase in length of regenerated tissue when it had replaced 62 per cent of the 15 mm. removed. In Experiment II the operated series had nearly completed the process of regeneration on the thirtieth day after the operation when 56 per cent of the amount removed had been replaced. This incomplete regeneration was also observed by Ellis. The irregularities in the rate curves may each be associated with some irregularity in the external conditions to which the series was subjected. Experiments I and III afford examples of an irregularity in food supply. The tadpoles of Experiment I were starved for four days, during which time the regenerating part of the tail shrank .5 mm. The irregularity in the food supply during Experiment III is of a different nature. The series was fed upon Oedogonium until the rate of regeneration was near the end of its interval of rapid decrease, then Spirogyra was given for two days. At the end of this period it was found that the rate, instead of having continued to decrease as it usually does at this time had increased slightly. During the following three days no food was given to the series and the rate of regeneration decreased to almost zero. In Experiment II (Table III and Fig. 2) the period between May 11 and May 15 included a cold Sunday, during which the laboratory was not heated. The rate for this period was much less than that for the following period. The irregularities occurring late

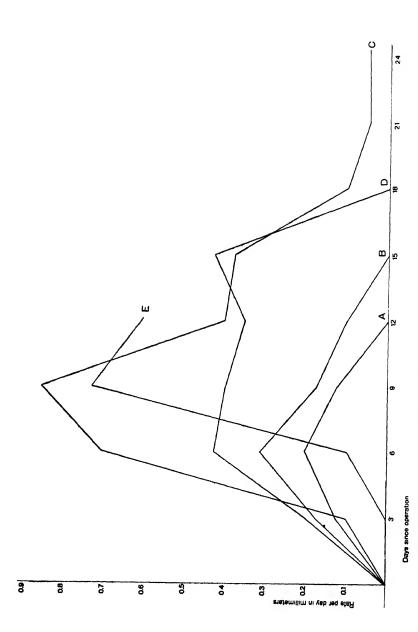


Fig. 5 Comparison of rates of regeneration from different levels. Data from Ellis ('99) A, Curve of the senes with 3 mm. of tail removed. B, Curve of the series with 5 mm of the tail removed. G, Curve of the series with 10 mm of the tail removed. D, Curve of the series with 15 mm of tail removed. E, Curve of four individuals of the series with 20 mm. of the tail removed.

in Experiment IV have been explained as due to individual variations after the series was reduced to eight, five and finally two tadpoles. The only example of a change in rate because of an

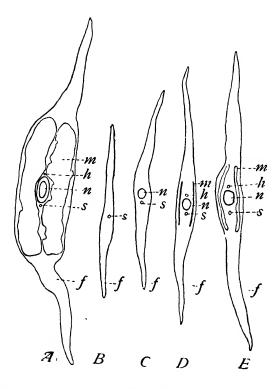


Fig. 6 Diagrams of the cross-sections of tadpole's tail. A, Cross-section of the uninjured tail. B, Cross-section of the new tail after three days' regeneration. C, Cross-section of the new tail after five days' regeneration. D, Cross-section of the new tail after ten days. E, Cross-section of the new tail after fourteen days' regeneration.

n = notochord
 m = muscle
 f = fin
 h = dorsal aorta
 s = nerve chord

irregularity in the aëration of the water is in the last period of Experiment I. The water on the tadpoles was not changed for seven days, and at the end of that time the regenerated tail had shrunken .2 mm. and the animals all showed signs of ill health.

It is to be noted that, although the length of the four intervals of change in rate of regeneration represented by the data, vary somewhat in the four experiments recorded, and although the rate is subject to variations occasioned by such factors as food, temperature, age and condition of the water; the four kinds of rate changes are plainly evident in each experiment, they follow each other in the same order, and each interval of change has the same relative duration in each experiment. Thus the first interval of low rate is short, it is followed by a longer interval of rapidly increasing rate. The third interval is not so long as the second, but longer than the first, it always has a rapidly decreasing rate. The fourth interval is longer than all of the others and has a gradually decreasing rate.

#### VOLUME

It was thought very desirable to find the rates at which volume was added to the regenerating tail. Several methods were tried with indifferent success. When the tail was weighed in air evaporation caused the weight to change faster than the scales could be read. The weighing of the tails in water was entirely inaccurate because the tail was too frail to be dried before it was weighed, and in the younger stages of regeneration the water adhering to it weighed as much as the tail. An examination of Table VI

TA	RI	F	VI
1.0	DI.	·Ľ	V 1

Days after operating	 	- • • • • •	 3	5	7	10	12	14	17
Volumes of tails at each stage in				I .	1	1		3.06 3.70	

will make this statement more comprehensible. The results given in this table were obtained in the following way. The tails prepared for the histological study (Experiment IV) were all carefully dehydrated at the same time, and then imbedded in paraffin likewise at the same time to insure similarity in the treatment with each reagent. Each tail was mounted in serial sections of

known thickness. Every fiftieth section was drawn with a camera lucida, and the area of each drawing determined with a planimeter. The table gives the volumes in cubic millimeters, as computed from these section and drawings.

An examination of these data make it evident that the maximum rate of increase in volume is near the tenth day; after this time the rate is much lower, but the irregularity of the data makes it impossible to say anything definite about the decrease in rate.

### DISCUSSION

A glance at the data is sufficient to reveal a general similarity between the changes in the rate of regeneration of the tadpole's tail and the changes in the rate of growth throughout the life of an animal. The rate of regeneration and of ordinary growth decreases from a maximum, attained soon after the process begins. The decrease is rapid at first, and then becomes gradually slower and slower. Minot ('08) conducted an elaborate series of experiments upon the rate of growth in rabbits, guinea-pigs and man, and expresses his results graphically by constructing curves showing the per cent of increment per day, throughout the life of the animal. These curves when based upon the rate changes, either from the time the egg is fertilized, or from the time the animal is born, show that the rate of growth undergoes four different kinds of change, which corresponds in general with the four intervals of change in the rate of regeneration. Minot does not emphasize the first two changes in the rate of growth, and they have no place in his discussion of the subject. In his study of the rate of growth, beginning with the fertilization of the egg, he remarks upon the fact that during the cleavage period there is no increase in weight. Also he makes frequent reference to the fact that it is sometime after birth, three to eight days, before the rate is at its maximum. In either case the initial interval of low rate is relatively shorter in the growth curve than in the curve of rate of regeneration. The second interval in the rate of growth is probably very short. It is combined with the first in all the figures given by Minot. It is in the regions of decline that the curve of rate of regeneration most closely resembles the curve of growth. In each the rapid decline from the highest point becomes more gradual as the curve approaches the base line, that is the rates at which growth and regeneration proceed decrease less rapidly as they approach their completion. Minot recognizes two factors controlling the rate of growth: (1) The tendency of undifferentiated cells to multiply; (2) the tendency of undifferentiated cells to differentiate into cells that do not multiply. The former is preponderant when the rate is high, the latter begins to influence the rate when it is at the maximum, and increases in its effect until it entirely overcomes the animal's power to grow.

The general similarity in the course of change in the rate of regeneration and the rate of growth, probably means that there is at least a general likeness in the factors controlling the two processes.

There are two possible explanations for the low rate in the initial interval of regeneration: (1) It may be that the shock given the animals by the operation makes them too weak to begin regeneration at once; (2) It may be that some time is consumed immediately after the operation in the formation of an embryonic tissue which serves as a basis for subsequent regeneration. The exact nature of the shock incident to such an operation, and its effect upon the tadpoles, can only be conjectured. It probably includes: (1) Certain local effects, as a bruising of the flesh on the line of operation, and the irritation of the cut by the water or by micro-organisms in the water; (2) Constitutional effects, as the loss of blood, the nervous shock due to pain inflicted by cutting the central nerve cord, and the waste of muscular energy, consumed in violent attempts to swim with the stump of a tail, to which the animal is not accustomed. The last two suppositions have nothing better to substantiate them than the actions of the tadpoles during and following the operation. When they are being measured tadpoles will not lie in one position for more than a few seconds. The operation causes them to give a sudden start, after which they lie relaxed and motionless for from twenty to thirty seconds. However, when they are placed in a dish of water

they quickly revive and wriggle violently until exhausted. The most specific evidence in favor of the view that the rate of the initial period is low, because the animals during that time, are laboring against the inhibiting effects of the shock of operation, lies in two facts: (1) The greater the amount removed from the tail of a tadpole of given size, the longer the interval of slow regeneration preceding the interval with a maximum rate; in other words, the more severe the operation the more concave the curve, from its beginning to its greatest height; (2) if the amount of tail removed is too large the animal dies soon after the operation. Fig. V shows the curves of rate drawn from the data, collected by Ellis in his "Experiment 4." The tadpoles of this set were 37-42 mm. long. Three millimeters were removed from each animal in one series; 5 mm. from those in another; 10 mm. from those of a third; 15 mm. from the fourth and 20 mm. from the fifth. The curves for the series with 3 and 5 mm. removed show little or no inhibition in the rate during the first period. The curve for the series from which 10 mm, were removed shows that the rate at the end of the first period is very little below what it would have been, had it increased uniformly from the time of operation to the time of maximum regenerating speed. curve for the series with 15 mm. removed shows that the rate in the first period is very low in comparison with that attained during the second period. The maximum rate for this series was not reached until three days after it had been reached by the animals in the three preceding series. Eighty per cent of the series, with 20 mm. removed died soon after the operation. four that did survive did not begin to regenerate until after the third day. On the ninth day they reached their maximum rate, but the new tails that were being formed were stunted and crooked.

However well these facts support the idea that the shock of operation is responsible for the low rate in the initial period, they are not opposed to the view that time is spent, previous to the beginning of rapid regenerations, in building up a layer of embryonic tissue, which serves as a basis for the subsequent regeneration. Sections of a tail, after it has been regenerating for three days, show that all of the cells in the new organ are undiffer-

entiated and embryonic in character. There are three possible sources one or more of which may have contributed to the production of these undifferentiated cells.

- I If totally undifferentiated cells are present in the tail, those in the immediate vicinity of the injury may have multiplied and formed a layer of cells over the surface of the wound.
- 2 There are in the tadpole tail many cells of a partially undifferentiated character whose normal functions are to continue the growth and carry forward the repair of the special tissue to which they belong. The cells of the chorda epithelium of the notochord and the large nucleated cells in the connective tissue are examples of this type, but without doubt the cells of the basal layer of the ectoderm are the most numerous and most active in this category. Barfurth ('03) describes these ectoderm cells as dividing and spreading over the cut end of the tail. quite probable that the first layer of cells which covers the wound is from the ectoderm; but it is also evident from the sections studied that the large nucleated cells of the connective tissues as well as those of the chorda epithelium produce undifferentiated cells of the kind destined to become the connective tissue and the core of the notochord, before the rate of regeneration has reached the end of its first low interval.
- 3 There is yet another way in which the undifferentiated cells of the regenerating tadpole tail comes into existence. It is described by Barfurth and others and applies in this connection to muscle tissue only. For present purposes it is sufficient to refer to it as a process, akin to budding, whereby undifferentiated cells called sarcoblasts and destined to become new muscle fibers, are produced from a nucleus and a little protoplasm of an old muscle fiber.

It is evident that some time must be consumed in the production of this layer of undifferentiated cells. Later a relatively small number of cell divisions occurring throughout this layer of new tissue will cause masses of cells to be protruded beyond the healed surface of the wound. Such masses of cells are the beginnings of measurable regeneration. From the study of the histological changes during regeneration it is shown that

muscle appears later than ectoderm, notochord and connective tissue. A cross-section of the tail at an early stage of regeneration has the space, that is later occupied by muscles, filled with embryonic connective tissue. When the rate has reached its maximum the cells which form the muscle fibers appear, at the level of injury, in the part of the muscle region nearest to the ectoderm. Since muscle tissue does not begin to produce new cells until after the measurable regeneration is well begun, it is evident that it does not contribute to the formation of the first layer of cells which covers the wound; furthermore regions of the tail in which much of the cross-section is composed of muscle will be slow to complete this first wound-covering layer, and hence slow to begin rapid regenerations. The upper levels of the tadpole tail contain proportionally more muscle in cross-section than is found near the tip, so it is not difficult to explain the relation between the amount removed and the length of the initial interval of low rate upon the theory that the rate in the initial interval is low because time is spent immediately after the operation in building a layer of embryonic tissue over the wound.

A combination view of the slowness of regeneration in the first period is probably more sane. The low rate is perhaps inevitable because undifferentiated tissue must be present at the level of injury before rapid regeneration can proceed; but the supply of nourishment to the new cells and the general body tone of the tadpoles may be influenced by the depressed condition they are in after the operation.

Whether the formation of an embryonic tissue has anything to do with the low rate in the first period or not, it is true that by the end of a day and a half or two days in most cases, the wound made by the operation is covered with a thick layer of undifferentiated cells many of which are dividing. This condition is quite enough to account for the second region of the curve showing the rate of regeneration rapidly rising to a maximum.

The third region of the curve, that representing the rapid decrease in rate, is more difficult to explain; it is closely associated with the fourth region, the one of gradual decline. An examination of Chart I (Experminent IV) will reveal a correspondence

between the rapid decline in rate and the rapid differentiation of the materials proliferated during the time represented by the first and second regions in the rate curve. It is easy to understand how differentiation by decreasing the number of dividing cells will decrease the rate at which the regenerating tail becomes The larger part of this reduction in rate by differentiation comes immediately after the rate has been high, when there are a large number of cells that are capable of differentiating in the regenerating bud of the new tail. It is with the completion of this differentiation on a large scale, that the third region of the curve ends and the fourth begins. After a large per cent of the undifferentiated cells have been differentiated both the growth of the new organ by cell division and the reduction of its rate of growth by differentiation must proceed more slowly because (1) there are fewer undifferentiated cells to divide; and (2) there are fewer to differentiate. These two processes may go on for some time, the proportion of the undifferentiated cells to the differentiated ones becoming constantly less and less until finally the per cent characteristic of these animals at their present age is reached.

This agreement between the time of rapid decrease in rate of regeneration and the time of rapid differentiation of the new tissue is an evidence in favor of the view that the differentiation of these cells in the new tail is the cause of the reduction in the rate of its growth. However, it must be remembered that no satisfactory explanation has been given of the factors which cause cells to begin to differentiate. It is quite possible that the beginning of differentiation depends upon a reduction of the rate of cell division in which case some other factor, at present unknown, is responsible for the changes in rate of regeneration after the maximum has been passed.

#### SUMMARY

- In tadpoles of Rana clamitans (Latreille), 35-40 mm. long, the removal of aboout 50 per cent of the tail is followed by the regeneration of 56 to 62 per cent of the amount removed.
  - 2 The rate at which this amount is replaced is not the same

throughout the regenerative period. Four kinds of change in rate can be distinctly recognized.

- 3 The operation is followed by an interval of low rate, succeeded by one of rapidly increasing rate, then by one of rapidly decreasing rate and finally an interval in which the rate gradually approaches zero.
- 4 The first low period is explained by the combination of two factors, (a) the shock of the injury and (b) the formation of a cap of embryonic cells which is to serve as a basis for the more active regeneration.
- 5 The second or period of rapidly increasing growth is the one in which practically all the cells in the new part are undifferentiated and are rapidly dividing.
- 6 The third and fourth periods are explained by the appearance of differentiation which lessens the number of dividing cells.
- 7 The curve representing these changes in the rate of regeneration agrees in general features with the curve of growth as described by Minot and others.
- 8 The above points strongly support the view that the essential factors controlling growth and regeneration are similar.

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# THE RELATION OF THE AMOUNT OF TAIL RE-GENERATED TO THE AMOUNT REMOVED IN TADPOLES OF RANA CLAMITANS<sup>1</sup>

# MAX MAPES ELLIS

WITH THREE FIGURES

## INTRODUCTION

The experiments furnishing the data employed in these observations were made at the Zoölogical Laboratory of Indiana University at Bloomington, during the years 1907–1908, on tadpoles of Rana clamitans (Latreille), and are a detailed study of a problem presented in the Biological Bulletin, April, 1908. In this report it was stated that, "the rate of regeneration varies not only directly but proportionally with the distance the cut is removed from the tip of the tail." This proportional relation was between the length of the part of the tail removed and that regenerated at the end of twelve days. The present experiments ascertained the relation between the amount removed and that regenerated at various intervals throughout the period of regeneration under several sets of conditions. The results may be stated in brief as follows:

- I After a certain percentage of the amount removed had been regenerated, regeneration ceased entirely. (This percentage was always less than one hundred.)
- 2 The same percentage of the part removed was regenerated by all tadpoles maintained under uniform conditions and of the same age, whether the amount removed was large or small; that is, the amount of regeneration at the time regeneration ceased was proportional to the amount removed.

<sup>&</sup>lt;sup>1</sup> Contribution from the Zoölogical Laboratory of Indiana University, No. 106.

- 3 The time elapsing between the operation and the cessation of regeneration varied with the amount removed.
- 4 The amount regenerated throughout the regeneration period was influenced by temperature.

The only regeneration considered was that of the tail; hence the term "regenerated" refers to the regenerated tail. The comparison made was that of length, the length of the regenerated part being compared with the length of the part removed.

#### FACTORS INFLUENCING REGENERATION

Throughout the experimentation an effort was made to consider all of the factors which might influence the amount of regeneration. These were generally controlled directly, but if this were impossible, they were reduced to a constant by the introduction of a "control series" of uninjured tadpoles whose reactions to the factors of the experiment were noted. The factors of largest importance are here considered.

- I Environment. The animals were kept in glass berry dishes containing about 150 cubic centimeters of water. These were of a uniform size and shape allowing each individual equal freedom and air. The dishes were covered with panes of window glass so arranged as to permit the free circulation of air between them and the top of the dishes. In Experiments 2 and 3, the groups of tadpoles were kept in large glass battery jars.
- 2 Food. Three kinds of food were used: fresh Spirogyra, raw beef and a combination of both. Some series were not fed at all. When Spirogyra was used, more was placed in the dishes than would be eaten before the next feeding time, so that there would be an excess of food always present. As the food was not the same for all experiments, that given in each case is stated at the beginning of the record of the experiment.
- 3 Temperature The temperature in Experiments 1, 4, and 9, was not controlled directly, but the average temperature of the water in the dishes was taken as a standard temperature for the experiments. The remaining experiments were subjected to definite temperature conditions. The jars containing the tadpoles

of Experiments 2 and 3 were almost submerged in water of a known temperature which transmitted a very constant amount of heat to the water within the jars. The dishes in which Experiments 6 and 10 were conducted, were placed in a chicken incubator; and those of Experiments 5, 7, and 8, in a chicken brooder. Both incubator and brooder gave very constant temperatures by means of their automatic temperature regulators.

- 4 Light Light was taken as a constant in these experiments as both the brooder and incubator had glass doors which allowed the animals within them the same light as those kept upon tables in the room. No animal received direct sunlight.
- 5 Age There was no definite way of obtaining the absolute age of any of the tadpoles except those used in Experiment 10, which were hatched from the eggs in the laboratory. Because of this fact, body length and tail length were used as units in the determination of the relative ages of the various individuals. It was assumed that tadpoles of the same species, collected from the same part of a given pond, and of the same body measurements were of about the same age. There must, of course, be some exceptions to this calculation of age; but the uniformity of results and data show that there was probably little error from this source.
- 6 Individual Variation The effect of individual variation was eliminated as far as possible by the use of large numbers of tadpoles. The deviation of any one animal from the mass is easily noted from the individual data.

## NATURE OF OPERATION AND MEASUREMENTS

The method of removing a part of the tail was the same for all animals used. A tadpole to be operated upon was placed on a smooth block of parraffine with its tail evenly spread and the body covered with a piece of wet cloth. The amount to be removed was then measured with dividers and the cut made at right angles to the notochord by a single stroke of a razor. Only those animals with a clean even cut were used. In determining the amount to be removed the measurements were always made from the tip of the

tail cephalad. The piece removed was immediately measured under a microscope with an ocular micrometer and the exact amount removed ascertained. The measurements of the regenerated parts were also made with an ocular micrometer, the living tadpole being placed upon a glass slide. The body length and tail length were taken with dividers and a steel rule.

The significance of certain terms used is as follows:

- "Amount removed" is the length, in millimeters, of the part of the tail removed.
- "Amount regenerated" is the length, in millimeters, of the part regenerated.
  - "Per cent regenerated" is the per cent of the amount removed.

#### DESCRIPTION OF EXPERIMENTS

# Preliminary Experiment<sup>2</sup>

The results of this experiment were mentioned in the introduction of this paper as pointing out the present problem. The table of averages is repeated here. The term "rate of regeneration" refers to the amount regenerated in millimeters, divided by the number of days during which the regeneration occurred. This rate of regeneration divided by the amount, in millimeters, removed gives the quantity termed "proportional rate."

TABLE 1 AMOUNT RATE OF PROPORTIONAL. REMOVED REGENERATION RATE mm.mm. 0.034 7 0.24 10 0.34 0.034 0.43 0.033 13 0.19 0.027 0.26 0.026 10 0.026 13 0.34

This table shows that the absolute rate varied with the amount removed, and that this variation was proportional to the amount removed. Two sets of tadpoles were used; hence the two divi-

<sup>&</sup>lt;sup>2</sup> Biological Bulletin, April, 1908.

sions of this table. The average body length of the animals used in this experiment was 40 millimeters. The measurements were made on the twelfth day after the operation.

# Experiment 1. Temperature, 74° F. Food, Spirogyra

Sixty tadpoles, body length 37-42 mm., were divided into three series—A, B, and C,—of twenty individuals each. The injury given Series A was the removal of 7 mm. of tail; Series B, 10 mm.; Series C, 13 mm. This experiment was maintained under the same conditions as the preliminary experiment just mentioned, which it was intended to duplicate. Twelve days after the operation, the amounts of regeneration were measured. The average amounts regenerated are given in Table 2.

TABLE 2

	AMOUNT	AMOUNT	PER CENT
	REMOVED	REGENERATED	REGENERATED
Series A	7	2.66	38
Series B	. 10	3.68	37
Series C	13	4.81	37

The per cent regenerated by each series during the twelve days of this experiment was approximately the same, although the amount removed in each case was different; that is, the length of the regenerated part was proportional to the amount removed. This experiment confirms the preliminary experiment as regards proportional regeneration, but it is obviously incomplete, since i states the relation between the amount removed and that regenerated at but one time after the operation, namely the twelfth day.

# Experiment 2. Food, Spirogyra and Raw Beef

This experiment was designed to ascertain the relation between the amount removed and that regenerated at some other time than the twelfth day after the operation. The effect of temperature on the amount of regeneration was also noted.

One hundred and sixty tadpoles, body length 37 to 42 mm., were placed in four large glass battery jars, forty in each jar. These

four jars (designated as Series D, E, F, and G) were kept at the following temperatures:

Series D	 76
Series E	
Series F	 57
Series G	 47

Ten tadpoles in each series were left uninjured, while the remaining thirty were operated upon thus: 2 mm. were removed from the tails of ten; 5 mm. from another ten; and 10 mm. from a third ten. At the end of five days the animals of Series D showed a distinct regeneration; accordingly, measurements of all the series were taken. No attempt was made to keep individual records, but the measurement of every animal in each series was used in computing the averages.

Series F and G (57 and 47° F.) showed no regeneration at the end of this first five days. The wounds were healed and the animals as active as when first collected, but there was no new tissue to be measured. On the other hand, however, both Series D and E had regenerated. The average regenerations are given in Table 3.

TABLE 3

	TEMPERA-	AMOUNT	5 DAY RE	GENERATION	12 DAY REGENERATION		
	DEGREES F.	REMOVED	MOVED Amount		Amount	Per Cent	
	:	mm.	mm.		mm.		
ſ	76	2	1.0	50	1.0	50	
Series D $\dots$ $\left. \left.	76	5	1.8	36	2.5	50	
{	76	10	2.4	24	4.9	49	
1	66	2	0.7	35	0.7	35	
eries E {	66	5	0.8	16	1.7	34	
	66	10	1.0	10	3.2	32	
	57	2	•	0	0	0	
eries F	. 57	5	0	•	0	0	
	57	10	0	0	0	0	
	. 47	2	0	0	0	0	
Series G	47	5	0	0	0	0	
	47	10	•	•	•	0	

From these averages it is to be seen that the relation of the amount regenerated to that removed is clearly not a proportional one at the end of five days, as was the case at the end of twelve days in Experiment 1. All of the series were continued until the twelfth day after the operation, a second set of measurements being taken at that time. Series F and G again showed no regeneration. All of the tadpoles in these two series were still as active as any of the others in the experiment, but the condition of the tail externally was precisely the same as when observed on the fifth day. There were no deaths in any of the series during the twelve days they were maintained and the general health of all the tadpoles seemed good. As all of the other conditions of the experiment were the same for Series F and G as for Series D and E, which did regenerate, this lack of regeneration was ascribed to the effect of temperature upon the processes producing regeneration.

Series D and E both made accretions to the amount regenerated during the seven days intervening between the fifth and the twelfth, excepting the first set in each series from which but two millimeters had been removed, which remained constant. was noted in Experiment 1, the per cent regenerated by the various sets of each series during the twelve days was approximately the same, showing the amount regenerated to be proportional to the amount removed at the end of the twelfth day. per centum regenerated, however, was not the same in Series D as in Series E, the latter being about fifteen per cent less than the former. This difference was evidently due to the lower temperature at which Series E was kept, as the other factors were the same. Three things are evident from the data of Experiment 2. (1) Temperature affects the amount of regeneration, other factors being constant. (2) There is a temperature limit below which tadpoles live but do not regenerate the tail. (3) The percentage of regeneration is not proportional at all times throughout the period of regeneration. Measurements of the control series show that the tadpoles did not grow during this experiment.

## Experiment 3. Food, Spirogyra and Raw Beef

Some months after the conclusion of Experiment 2, another set of tadpoles were subjected to the same conditions used in Experiment 2. Two other series at temperatures not used in Experiment 2 were added. One of these was maintained at 96° F. and the other at 103° F. Table 4 is made up from the data collected in this experiment. The measurements were taken but once, the twelfth day after the operation.

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1	TEMPERA- TURE ° F.	AMOUNT REMOVED	AMOUNT REGENER-	PER CENT REGENER- ATED
		mm.	mm.	
<b>(</b>	66	2	0.6	30
Series H	66	; 5	0.6	32
	66	10	3.0	32
	76	2	0.9	45
Series J	76	5	2.2	44
{	76	10	4.5	45
Series K	57	all animals ac	tive; no deaths	no regenera
Series L	47	all animals ac	tive; no deaths;	no regenera-
Series M	96	all animals de	ead in 24 hours	after opera-
Series N	103		lead in less th stion	an 12 hour

It was hoped that tadpoles would live at a temperature higher than 76° F. but unfortunately, the two temperatures above 76 were too high to sustain life. There is the same proportional relation between the amount removed and the amount regenerated at the end of the twelfth day, as shown by these data as has been previously noted. The per cent regenerated in this experiment was lower than that regenerated in Experiment 2, which shows a variation at some point between the conditions controlling the two experiments. A possible cause of this difference may be the influence of the season upon tadpoles' ability

to regenerate; for the animals used in Experiment 3 were collected in February, while those used in Experiment 2 were taken early in December.

Experiment 4. Food, Spirogyra. Temperature, 78° F.

In this experiment the relation between the amount removed and that regenerated was obtained every third day throughout the period of regeneration.

Five series, O, P, R, S, and T, were established, twenty tadpoles in a series, body length 37 to 42 mm.; and a control series of forty other uninjured tadpoles arranged. Series O was injured by the removal of three mm. of tail from each individual; Series P, 5 mm.; Series R, 10 mm.; Series S, 15 mm.; and Series T, 20 mm. The regenerated part was measured every three days and the whole experiment maintained for thirty two days. Individual records of the amount regenerated by each tadpole were kept. (See Tables 9, 10, 11, 12, and 13. Also Fig. 1.)

It was found that Series T behaved differently from the other series of this experiment, accordingly it is considered separately. At the end of the first three days after the operation, almost half of the tadpoles in Series T (20 mm.) were dead, and those living were sluggish and weak. The survivors had not regenerated any of the lost tail, the wound not being even healed over in several cases. By the end of the sixth day, seven more of this series had died and the death of another individual on the eighth day reduced the number of living to four. These four continued to live and each one regenerated a part of the removed tail. This regenerated tail, however, was not normal, being twisted and very irregular of out-It is evident that with a death rate of 80 per cent and abnormal regeneration by those four surviving the operation, the injury was too severe in Series T. The final amount regenerated by the four survivors of this series averaged 20 per cent of the amount removed.

The average regeneration of the four remaining series on the third day after the operation were:

												•													1	MM.	•
Series	0										 		٠.	 		٠.	 									.4	ŀ
Series																											
Series	R	٠.	٠.		 	 																				.6	,
Series	S.			 	 																	 	 			0	

The differences between these amounts are slight, but subsequent observation shows them to be significant. The relation between the amount regenerated at this time and the amount removed is not a proportional one. The series of particular interest at this time is Series S (15 mm.) which had no measurable regeneration at the end of the third day. The regeneration of this series was recorded as zero because the amount regenerated was too small to be accurately measured; there was, however, regeneration present at the end of the third day, as in most cases a distinct film of new tissue was visible. All of the wounds were healed at this time. By consulting Table 13, the amount of regeneration can readily be followed.

All of the series, including Series T, had definite regeneration at the end of the sixth day. These amounts were arranged according to the amount removed, although not proportional to it. The ninth day measurements bear the same relation to each other, that is, the amount regenerated varied in the same direction as the amount removed, but was not proportional to it. The ninth day percentages, however, were distinctly nearer the same than those of the third or sixth day. This places the relation of the amount regenerated to that removed, much nearer the proportional. The amount regenerated by Series O at this time was 1.4 mm., 44 per cent of the amount removed. Subsequent measurements show that this series never regenerated beyond this per cent, which remained constant throughout the remainder of the experiment. On the twelfth day Series P also ceased regenerating, its final amount of regeneration being 2.3 mm., 44 per cent of the amount removed. Thus, on the twelfth day after the operation, two of the series, O and P, had reached a limit of regeneration, which was proportional to the amount removed. The other two series, R and S, although not having regenerated the same percentage as that regenerated by Series O and P, were not far below it. The

next series to cease regeneration was Series R, on the fifteenth day after the operation. The final amount regenerated by it was 5 mm., 48 per cent of the amount removed. Series S stopped regenerating on the eighteenth day with an amount of 7.2 mm., 48 per cent of the amount removed. From the eighteenth day until the close of the experiment all of the series remained constant and did not regenerate beyond the limit they first reached. Because of this fact, the curve for all of the series from this time on are straight lines parallel to the base of the plot.

An examination of Fig. 1 shows each curve to be composed of four distinct regions. The first, one of slow regeneration, lasts for the first three days after the operation; a second, of rapid regeneration, which is of several days' duration; a third, of regeneration distinctly slower than that of the second region; and a fourth, in which the regeneration gradually ceases. At the end of the fourth region, the regeneration probably ceases entirely; although, there is some evidence of very slight regeneration for many days after the apparent cessation of regeneration. The average amounts regenerated by the various series up to the time when regeneration ceased, are to be found in the following table:

TABLE 5

	AMOUNT REMOVED	FINAL AMOUNT REGENER- ATED	FINAL PER CENTUM REGENERA- ATED	
	mm.	mm.		
nes O	3.2	1.4	44	
s P	5.2	2.3	44	
es R	10.4	5.0	48	
es S	14.8	7.2	48	
es T (16)	20.2	died	•	
es T (4)		4.1	20	

The two Series T mentioned in the above table, are two divisions of that series; the numbers in parenthesis after each refer to the number of individuals. From this table it is to be seen that the amount of regeneration at the time regeneration ceased, was pro-

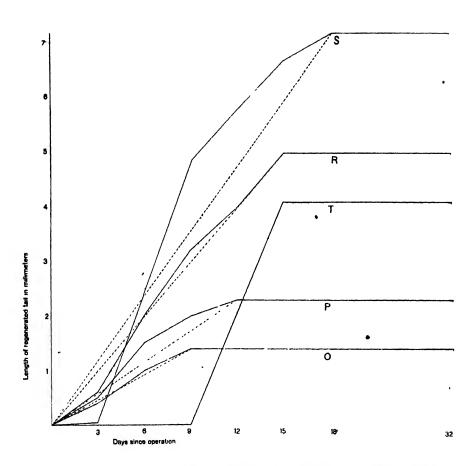


Fig. 1. The curves represent the amount of regeneration for Series O, P, R, S, and T, Experiment 4. The abscissa represents time, and the ordinate, the length of the part regenerated in millimeters. The dotted lines are the paths of regeneration, had the amount of regeneration been laid down at a uniform rate, throughout the period of regeneration. They give a standard to which the curve of amount may be compared.

Tables 9, 10, 11, 12 and 13 give the individual records for the tadpoles of series 0, P, R, S, Experiment 4.

Table 13 gives the average percentages regenerated for all of the series of Experiment 4, throughout the period of regeneration.

portional to the amount removed. The time required by the various series to regenerate these amounts, that is, the length of time during which regeneration took place, was not the same for these several series, but varied in the same direction as the amount removed.

From the experiment as a whole three things are evident: (1) The regeneration of the part removed is not complete; for in every case the regeneration ceased after about 46 per cent of the amount removed had been regenerated. (2) The amount regenerated at the time that regeneration ceased, which was the complete amount laid down during the period of regeneration, varied directly as the amount removed, that is, was proportional to the amount removed. (3) The length of time through which the period of regeneration extended, was not the same for the various amounts of injury; but it grew longer the greater the amount removed.

In this connection, the behavior of the series with 2 mm. removed in Experiment 2, is to be considered. Under that experiment, it was noted that the amount regenerated by the individual with an injury of 2 mm. was the same on the twelfth day as on the fifth day after the operation; in other words, in this case there was no additional regeneration after the fifth day. The smallest injury inflicted in the present experiment was the removal of 3 mm., that in Series O. This series ceased regenerating on the ninth day after the operation. Comparing these two sets of tadpoles, it is seen that, these with 2 mm. removed reached the end of their period of regeneration some four days earlier than those of Series O with 3 mm. removed. Although this comparison is not strictly accurate, it does show that the time through which regeneration takes place, the duration of the period of regeneration, varies in the same direction as the amount removed.

The control series of Experiment 4 showed no growth during the time of this experiment.

## Experiment 5. Temperature, 80° F. Food, Raw Beef

One hundred tadpoles, body length 37 to 42 mm., were operated upon in the same manner as those of Experiment 4. The experiment was maintained for thirty days in a brooder at 80° F. The results agree exactly with those obtained in Experiment 4. All of the series, except those with 20 mm. removed, ceased regenerating after about 47 per centum of the amount removed had been regenerated. All of the tadpoles of Series Y (20 mm.) died during the first few days after the operation.

TABLE 6

	AMOUNT REMOVED	AMOUNT REGENER- ATED	PER CENTUM REGENER- ATED	DAYS OF REGENER- ATION
	mm.	mm.		
Series U	3.1	1.5	48	9
Series V	5.0	2.4	48	11
Series W	10.1	4.9	49	15
Series X	14.9	7.4	49	19
Series Y	20.1	died		

<sup>·</sup> Experiment 6. Temperature, 82°F. No Food Given

Twenty-four small tadpoles, body length 18 to 20 mm., were divided into two series, AA and BB. The average amount removed in Series AA was 2.5 mm. of tail; in Series BB, 5.2 mm. (See Table 14.)

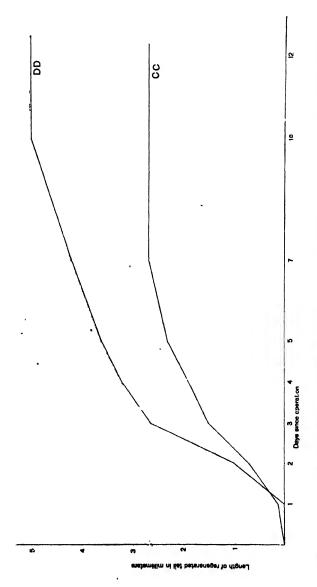
Series AA ceased to regenerate on the fifth day after the operation, with an amount of 1.4 mm., 55 per cent of the amount removed; Series BB continued to regenerate until the seventh day. Its final amount of regeneration was 2.9 mm., 52 per cent of the amount removed. The only point of difference between these results and those of Experiment 2, since the amount regenerated was proportional to the amount removed, is the absolute per cent of regeneration; this is higher in the present experiment. Two things probably contribute to this difference, the tadpoles are younger and the temperature is higher. Both of these factors increase the rate of regeneration.

As originally planned, it was intended that Experiment 6 should supply some data concerning the surface and the volume of the regenerated parts, but the difficulties encountered in computing these values by the method chosen were so great as to render the data too inaccurate to be of value. It will be noticed in Table 14, which gives the individual data of this experiment, that various individuals do not have complete records. These were killed to be used in the volume and surface calculations. By the plan adopted, camera lucida drawings were made of the regenerated parts as whole and also of two typical cross-sections of the same. From these the other values were calculated; the results, however, very unsatisfactory.

## Experiment 7. Temperature 82° F. No Food Given

Eighteen tadpoles, body length 27 to 31 mm., were divided into two series, CC and DD. From Series CC an average amount of 5.4 mm. was removed; from Series DD, 9.8 mm. This experiment was to ascertain two things: (1) the relation between the amount removed and that regenerated during the first few days after the operation; (2) the average per cent regenerated by tadpoles of this size. (See Tables 15, 16 and 17. Also Fig. 2.)

Fig. 2 shows the same regions in each of its curves as those found in the curves of Experiment 4. There are, as before, four distinct regions. A first, of slow regeneration; a second, of rapid regeneration; a third, of slower regeneration than the second; and the fourth, in which regeneration gradually ceases. Both series regenerated 50 per cent of the amount removed, making the relation between the amount removed and that regenerated, a proportional one. The final average amounts regenerated by these two series here follow, to wit: Series CC, 2.7 mm., 50 per cent; Series DD, 5.0 mm., 50 per cent. Series CC ceased to regenerate on the sixth day after the operation; Series DD, on the tenth. The regeneration in these two series ceased when the amount removed was proportional to the amount regenerated; and the time consumed in regenerating this amount varied with the level of injury. The point of largest interest in this connection is the first part of the curves of the two series. Neither curve showed great regeneration during the first two or three days after the operation; the rapid regeneration coming several days after the operation in both cases. This experiment was maintained twelve days.



Abscissa, time in days; ordinate. length of the part regenerated. This is the most accurate plot made, as the measurements were taken daily for the greater part of the curve. Fig. 2. The curves represent the amount of regeneration for Series CC and DD, Experiment 7. Table 14 gives the averages and individual data for the two series, Ad and BB. Table 15 gives the averages of Series CC and DD.

## Experiment 8. Temperature, 82° F. No Food Given

This experiment was made with twenty tadpoles, average body length 45 mm. One-half of these, Series EE, was injured by the removal of an average amount of 5.1 mm. of tail; the other half, Series FF, 10.2 mm. The object of this experiment was to obtain additional data concerning the relation of the amount regenerated during the first few days after the infliction of the injury, to the amount removed. These tadpoles (see Fig. 3, and Table 18) also ceased regenerating before the entire amount removed had been restored. Series EE ceased to regenerate after having regenerated 2.3 mm., 47 per cent of the amount removed, on the ninth day. Series FF regenerated until the nineteenth day, when its average amount was 4.4 mm., 44 per cent of the amount removed. This experiment lasted twenty-two days.

# Experiment 9. Temperature 70° F. F.ood, Raw Beef and Spirogyra

Two series, Series GG and HH, of twenty tadpoles each, body length 37 to 42 mm. constituted this experiment. Its object was to ascertain the constancy of the regenerated part after regeneration ceased. From Series GG 5 mm. of tail were removed; from Series HH, 10 mm. Both series were kept on top of a table in a room whose average temperature was about 70° F., for forty-two days. Series GG ceased regenerating on the eleventh day with 40 per cent of the part removed, restored. Series HH did not regenerate after the nineteenth day, its final per cent regenerated being 39. These per cents remained the same until the experiment was discontinued.

## Experiment 10. Temperature, 74° F. No Food Given

The eggs, from which the animals used in this experiment hatched, were brought into the laboratory on April 10, 1908. The tadpoles hatched out six days later. When they were a week old, thirty-two, of an average body length of 12.2 mm., were chosen. Four series were used, a control of uninjured tadpoles and three

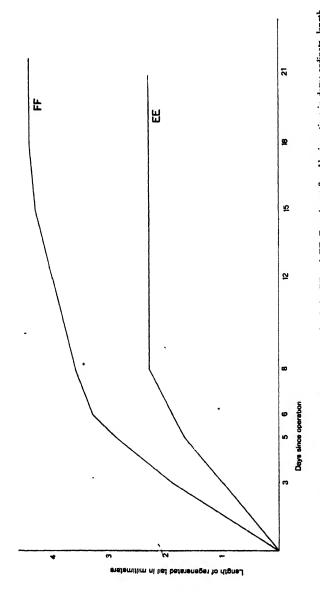


Fig 3. The curves represent the amount of regeneration for Series EE and FF, Experiment 8 Abscissa, time in days; ordinate, length of the part regenerated.
Table 16 compares the averages of Series EE and FF.

other series operated upon as follows: one-fourth of the tail was removed from eight; one-half of the tail from another eight; and three-fourths of the tail from a third eight: This experiment was unexpectedly brought to a close on the fourth day after the operation, and is mentioned here for but two things. (1) During the four days of the experiment some of the individuals of the series which had lost one-fourth of the tail, regenerated more than 100 per cent of the part removed. This is the only case in these experiments, in which all of the removed part was regenerated. It must be noted, however, that during these four days the control series grew from 12.2 mm. to 16 mm., body length. (2) Almost all of the animals with three-fourths of the tail removed, died.

### DISCUSSIONS AND CONCLUSIONS

### Temperature

Temperature was found to influence the amount of regeneration. Its effect is direct, and the amount of regeneration varies in the same direction as the temperature, between two thermal limits. In Experiments 2 and 3, tadpoles regenerated the tail at 66° F. but did not regenerate at 57° F. even though the other conditions of the experiments were the same. As the temperature factor was the only variable in these two experiments, the lack of regeneration at 57° F. must be ascribed to the effect of temperature upon the processes producing regeneration. This lack of regeneration at 57° F. is of particular interest since the animals did not die, although they did not regenerate. The minimum temperature at which regeneration takes place, although not absolutely determined, is between 66° and 57° F. for the type of tadpoles used. Considering the other temperatures in Experiments 2 and 3, it is seen that tadpoles regenerated readily at 76° F. while they died in less than twenty-four hours at 96° F. The temperature at which most of the experiments in this work were maintained, was 78° F. Tadpoles regenerated readily at that temperature. highest temperature used, at which regeneration took place, was 82° F. in Experiment 6. On the contrary, tadpoles were kept at 47° F., 19° below the lowest temperature at which they regenerated,

and they still remained as active as those kept at the higher temperatures, at which regeneration took place. It is also known, that tadpoles will live at even a lower temperature than 47° F.; they have been collected by the writer from ponds in which there was floating ice, and the water of which was certainly not above 38° F. Taking the temperatures as observed, however (57 to 96° F.), regeneration is absent, present and prohibited by death, in a gamut of forty degrees. The absolute thermal limits of regeneration, as found in these experiments, were 82° and 66° F., a range of but 16°. Between these limits the data collected show the amount regenerated to vary in the same direction with the temperature. This is particularly apparent in Experiments 2 and 3, but may be shown to be the rule for all of the experiments by cross-comparison.

Two conclusions were drawn from the data collected concerning the relation of temperature to the amount regenerated:

- There is a minimum temperature, below which tadpoles live but do not regenerate the tail. It lies between 57° and 66° F. for tadpoles, body length 37-42 mm.
- 2 Between 66° and 84° F., the amount of regeneration varies in the same direction as the temperature.

### Level of Injury

Spallanzani in 1769 made the following interesting statement concerning the relation of the level of injury to the amount regenerated, in the tadpole tail. He says: "If the whole tail, or very near the whole tail, be cut off, the tadpoles go to the bottom of the water and there lie down and perish. But if a lesser part be taken off, not one of them dies; and all without exception, recover what they lost. . . . . Nature observes the following laws in the growth of these reproductions. They are more considerable, when a great part is taken off; not so large after a lesser section and least of all when a very small bit has been cut off. The greatest length seems however, rather to take place, when the tail is divided in the middle, than when the section is higher."

The relations of the level of injury to the regeneration were found to be precisely those pointed out in the observations of Spallanzani. First as regards the level from which the removal of the tail pro-

duced a fatal injury. In Experiment 4 it was noted that Series T (20 mm.) had a mortality of 80 per cent; and that the four tadpoles which did survive the loss of 20 mm. of tail, regenerated abnormally. All of the tadpoles in the series with 20 mm. removed, died, in Experiment 5. Since tadpoles of the same size and subjected to the same conditions as these animals just mentioned, regenerated rapidly from the 15 mm. level, the 20 mm. level, in the light of the above statements, was considered the lowest level (that is the level nearest the tip of the tail) from which the removal of the tail produced fatal injury. Injury at the 20 mm. level, removed about 80 per cent of the tail, the average tail length of these tadpoles being 26 mm. The entire tail was removed from several tadpoles, independent of the experiments mentioned, and all of the animals so operated upon died within two or three days after the injury.

Consider now the levels at which regeneration took place. Beginning near the tip of the tail, injuries at all levels were followed by regeneration until the fatal level, 20 mm., was reached. The per cent of the part removed, regenerated from the various levels, was the same regardless of the level; that is, the amount regenerated was proportional to the amount removed. The time, however, consumed in regenerating grew longer, as has already been mentioned, as the level of injury approached the head. Although the length of the period of regeneration was greater for the regeneration from the higher (the more cephalad) levels; this period was relatively longer for the series injured at the lower (the more caudad) levels, making the rate of regeneration higher, the more cephalad the injury. Take for example the final amounts of Experiment 4 which are typical. Together with the time required by each for this regeneration, and the average rate per day, in millimeters, they may be grouped in the following table:

TABLE 7

	AMOUNT	FINAL AMOUNT REGENER- ATED	DAYS OF REGENERA- TION	AVERAGE DAILY RATE OF REGEN- ERATION
	mm.	mm.		mm.
Series O	3.2	1.4	9	0.16
Series P	5.2	2.3	12	0.19
Series R	10.4	5.0	15	0.33
Series S	14.8	7.2	18	0.40
Series T (16)	20.2	0	Died in 4	to 6 days
Series T (a)	20.2	4.1	15	0.27

From this table it is to be seen that although the per cent regenerated was the same, regardless of the amount removed, the rate of regeneration varied with the level of injury, as the level moved away from the tip of the tail. Precisely the same relation between the level of injury and the rate of regeneration has been found to obtain for the arms of the star-fish, Asterias, by King, 1808. She states: "The rate of regeneration is greatest from the disc and decreases directly towards the tip of the arm." Morgan, in 1902, stated that the regeneration from straight cuts across the tail of the fish, Fundulus, proceeded more rapidly from the higher In the recent paper of Stockard on the regeneration of Cassiopea xamachana, one finds the following observation: "The regeneration rate is fastest at the deepest level, and slower as the level nears the margin. The Medusa regenerates tissue faster the farther away from the periphery the cut is made, as though the more tissue removed the less uninjured body-surface remained to exert a retarding influence."

Thus the data collected concerning this relation of the amount and rate of regeneration from various levels of the same organ, agree in supporting the hypothesis that regeneration always proceeds more rapidly from the higher or deeper levels of the same part or organ.

The amount regenerated by the four individuals surviving the removal of 20 mm. of tail in Series T was added to Table 7, as

it showed the effect of extremely severe injury upon the rate of regeneration. The rate of regeneration of these four was lower than that of the Series R (10 mm.); this fact is probably to be accounted for by the effect or shock of the extreme injury. No conclusion, however, can be reached from these four animals alone.

Another interesting relation between the level of injury, and regeneration, is noticed in the length of the first period of regeneration, the one of low rate, in all of the experiments. This period is longer the higher the level of injury, that is, the greater the amount removed. It does not, however, increase proportionally with the level of injury. This difference in the length of this first period of regeneration may be due to one or both of two things: (1) The direct effect of the injury itself, that is, the shock of injury occasioned by the sudden loss of a quantity of blood and the overstimulation of the nervous system; that is, the sudden appearance of the abnormal factor, injury, in normal life; or (2) the delay due to the healing over of the surface of the wound. Either of these would prolong this first period of slow regeneration; the former, since the amount of injury, that is, the absolute amount of tissue removed, varies with the level of injury; the latter, since the area of the cross-section of the tail, hence the area to be healed over. varies with the level of injury. Data were not collected from which conclusions could be drawn concerning this point, but the histological investigations of Durbin on the early stages of regeneration of the tadpole, show the latter to be the more probable.

The following conclusions are taken from the data collected:

- I The level of injury first producing death was 20 mm. cephalad from the tip of the tail, tail length 26 mm.
- 2 The rate of regeneration varies as the level of injury until the 20 mm. level is reached.
- 3 The first slow period of regeneration increases in length the higher the level of injury.

## Relation of the Amount Regenerated to the Amount Removed

In both the experiment which suggested these investigations, and Experiment 1, it was noted that the amount regenerated at the end of twelve days after the operation, varied directly, that is,

was proportional to the amount removed. In Experiments 2 and 3, the same relation existed between the amount regenerated and that removed, at the end of the twelfth day as was noted in the two previous experiments; this relation, however, did not obtain at the end of the fifth day after the operation. Subsequent experiments gave two important facts in this connection. (1) Regeneration ceases before the part removed has been completely regenerated; and (2) the amount regenerated is proportional to the amount removed, regardless of the absolute amount removed, only at the time regeneration ceases. The tadpoles from which these data were collected did not grow while under observation, as shown by the control series. In but a single case did the tadpoles used regenerate the complete amount removed; these animals were but a week old and growing rapidly (Experiment 10). There is a very probable explanation for this incomplete regeneration on the basis of the state of development of the tadpole. Kammerer found that the rate of regeneration of tadpole tails varied not with the absolute age of the tadpole, but with its state of development. Suppose then, that there is a time (as there probably is) while the tadpole is very young, that it is capable of regenerating completely a part of the tail lost by injury. Data collected from observations on various animals show younger animals, as a rule, to regenerate more readily than older ones; that is, animals at a more advanced stage of development regenerate less rapidly. This fact was pointed out as early as 1786, by Broussonet in his observations on the regeneration of the tails of fishes. It is also known, that the adult frog, normally, does not regenerate. The tadpole, then, must lose its ability to regenerate in one of two ways: either suddenly, at some particular point in its life; or gradually throughout its larval life. The latter is the more probable, and is substantiated by the data collected. If the ability to regenerate be dependent upon the state of development it were possible then to establish a "regenerative coefficient" for the various ages of tadpoles; which coefficient would be the maximum per cent that the particular stage is capable of regenerating, under the best conditions for regeneration. This per cent, naturally, however, would not be regenerated; for various other factors also control the

per cent regenerated. In Experiments 2 and 3, for example, the various per centums, from zero to fifty, were regenerated by the several series, although the tadpoles comprising these were of the same age. This difference was due to the effect of temperature; and similarly other factors may be shown to influence the amount regenerated. A confirmation of the hypothesis, that the age or state of development does influence the amount regenerated and that the more nearly the adult condition is reached the lower the rate of regeneration, occurs in the following Table, constructed from the percentages regenerated in Experiments 6, 7, and 8, which were subjected to the same conditions.

TABLE 8

AVERAGE BODY LENGTH		PER CENT
	mm.	REGENERATED
Experiment 6	19	55
Experiment 7	29	50
Experiment 8	45	46

These comparisons are, perhaps, not absolutely accurate, since, as explained in the first part of this paper, body length had to be taken as an index of the age or state of development; but allowing for this inaccuracy, there are definite differences between the amounts regenerated by the tadpoles of various sizes under the same conditions.

At this point, the process of regeneration itself should be considered. Regenerated tissue is the direct product of cell division, and accordingly, the rate of regeneration is dependent largely upon the rate of cell division. According to Morgan's tension theory, the cell tends to divide until the external (that is, extracellular) pressure and tension are equal to the force within itself, causing it to divide. When these forces are equal, differentiation begins. Minot and others have shown that the more embryonic the cell, the more it tends to divide; in other words, the greater this force which prompts the cell to divide despite extra-cellular forces. The tail of a tadpole is an embryonic organ; hence, probably contains large numbers of embryonic cells. As previously mentioned, Kammerer found the rate of regeneration to vary with

the state of development of the tadpole. Taking the term "state of development" used by Kammerer, to mean the relative embryonic age of the tadpole, the larger the tadpole, that is, the greater its body length, the less embryonic the condition of the tail. Applying this to regeneration, take for example, the tail of a tadpole that has had approximately ten millimeters removed from it. By the very presence of injury, which has removed part of the tail, the cells on the exposed surface are given a chance to divide; as the pressure and tension at that point have been reduced. If the conditions of temperature, food, etc., are optimum, and the injury itself has not overwhelmed the animal (that is, such injuries as are fatal) these cells exposed will divide because of a lack of equilibrium of forces. They will also continue to divide until the extra-cellular forces are equal to the force producing cell-division. When this point has been reached, they will cease dividing, and differentiate. The data collected by Durbin on this point show two distinct regions in the period of regeneration as are to be expected from the above statements. (1) A region of rapid celldivision and no differentiation, which extends over the first part of the regeneration period; (2) a region of differentiation with little cell-division immediately following the first. The question comes at once, as to the per centum regenerated before cell-division ceases. This is dependent upon the state of development of the animal; for the amount of force producing cell-division varies with the relative embryonic age of the cells. This theory of regeneration is confirmed by the second fact mentioned at the first of this discussion, namely, that the final amount regenerated in series under the same conditions, by tadpoles of the same size, was always the same per cent of the amount removed, regardless of the level of injury. Consider, for example, two series of tadpoles of the same size and under the same conditions, one with 10 mm. removed, and the other with 5 mm. removed. The cells exposed have the same division potential, consequently, the same ability to overcome the extra-cellular forces inhibiting cell-division; and because of this fact, they will continue to divide until the same force is exerted against them. It is evident that the regeneration of two and one-half millimeters by both series would not produce

the same conditions of pressure and tension in the two sets of tails. The series with 10 mm. would probably have to regenerate 5 mm. to bring about a condition similar to that in the tail, that had regenerated two and one-half millimeters, but had lost only 5 mm. through injury. This is precisely the method of regeneration followed by all of the series observed. Each regenerated an amount proportional to that removed. This is to be expected in view of the last statement, for the regeneration would cease when the cells dividing had reached a point at which the extra-cellular force was equal to that force causing them to divide; and since these cells had equal regenerative potential to start with, the conditions of pressure and tension would only be equalized when the amount regenerated was proportional to the amount removed.

Another point in favor of this view of the cause of the proportional regeneration from various levels, is the sequence of the regions comprising the curves of regeneration. The first region, that of low rate of regeneration, is due to the immediate shock of injury and the healing over of the exposed surface; the second, of rapid regeneration, is the period during which the minimum resistance is offered to cell-division; the third, that in which the rate of regeneration suddenly decreases, is produced by the rapid cell-division of the second region, which has brought up the tension of the tail to a point almost equal to that, at which the extra-cellular forces are equal to those causing cell-division; the fourth and last region, that in which regeneration gradually ceases, is that in which the extra-cellular forces finally overcome cell-division.

### GENERAL SUMMARY

- I The tadpoles observed did not completely regenerate the part of tail removed.
- 2 The absolute amount regenerated was proportional to the amount removed, whether this was large or small; the per cent regenerated, by series under uniform conditions, was the same, if they were of the same relative age, regardless of the absolute amount removed.
- 3 The per cent regenerated, other conditions being the same, varied with the relative age of the tadpole.

- 4 The rate of regeneration increased directly with the level of the injury, as the level of injury moved cephalad from the tip of the tail. The removal of 20 mm. of tail constituted a fatal injury to tadpoles whose tail length was 26 mm.
- 5 Other factors being the same, the amount regenerated varied in the same direction as the temperature, between 66° and 84° F.
- 6 There is a temperature limit below which tadpoles will live but do not regenerate the tail. This limit was between 57° and 66° F.
- 7 There are four distinct periods of regeneration: (1) the first of slow regeneration during the first few days after the operation; (2) the second of rapid regeneration; (3) a third in which the regeneration is much slower than the second; (4) a last period in which the processes of regeneration gradually cease.
- 8 The time elapsing between the operation and the cessation of regeneration, that is, the period of regeneration, varies with the level of injury. The higher the injury the longer this period; though the period is relatively longer for the series operated upon at the lower levels.

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TABLE 9-Series O

CAT. NO.	BODY LENGTH	TAIL LENGTH	AMOUNT	3 DAY AMOUNT REG.	6 DAY AMOUNT REG.	9 DAY AMOUNT REG.	32 DAY AMOUNT REG.
704	41.2	25.9	3.1	0.4	1.2	1.3	1.3
708	40.3	25.6	3.0	0.4	1.0	1.2	1.1
715	39.0	25.0	3.5	0.4	0.9	1.1	1.2
716	37.8	24.3	3.0	0.4	1.0	1.3	1.2
720	41.0	26.0	3.1	0.4	1.0	1.2	1.2
723	42.0	27.3	3.0	0.4	1.1	1.4	1.4
729	40.0	26.0	3.0	0.4	1.1	I.2	1.3
735	37.5	24.5	3.0	0.4	1.0	1.2	1.3
741	41.0	25.6	3.0	0.5	1.1	1.2	1.2
743	41.6	27.2	3.5	0.3	1.0	1.4	1.3
751	40.0	26.0	3.5	0.4	1.1	1.5	1.4
756	37.2	29.3	3.5	0.3	1.0	1.6	1.4
760	43.3	28.5	3.0	0.3	1.1	1.1	1.2
769	37.0	24.6	3.4	0.5	1.1	1.2	I.2
777	38.5	25.0	3.5	0.5	1.1	2.2	2.2
779	40.8	26.5	3.5	0.4	1.0	1.3	1.3
781	37 · 9	24.7	3.6	0.5	0.9	1.7	1.7
787	37-4	26.0	3.5	0.4	1.2	1.6	1.6
789	37.5	23.9	3.0	0.4	0.9	I.2	1.2
791	40.5	26.0	3.0	0.4	0.9	1.1	1.2
erages			3.2	0.4	0.1	1.4	1.4

TABLE 10-Serses P

CAT. NO.	BODY	TAIL LENGTH	REMOVED	3 DAY AMOUNT REG.	6 DAY AMOUNT REG.	9 DAY AMOUNT REG.	I2 DAY AMOUNT REG.	32 DAY AMOUNT
706	37 8	24 0	5.0	0.5	1.4	2.0	2.0	2.1
711	40 7	26 5	50	0.5	• 1.5	1.8	2.0	2.1
713	43.1	27.5	5.0	0.4	1.4	1.7	1.9	2.0
719	42.3	27.0	5.2	0.6	14	1.8	2.3	2.3
725	40.6	26.1	50	06	13	1.8	1.8	2.0
726	41.0	26.5	5.1	0.5	1.5	2.0	2.2	2.2
736	43.5	28.0	6.0	05	16	<b>2</b> T	2.3	2.3
748	41.6	26.3	5.0	0.4	1.5	1.9	2.1	2.1
752	18.4	25.2	5.0	0.5	r.6	2 0	2.2	2.2
755	37.6	25.0	5.5	0.5	1.4	20	2.3	2.3
765	39.9	26.2	5.1	0.4	1.5	2.0	2.3	2.0
766	40.0	26.0	6.0	0.6	1.4	18	2.0	2.0
773	40.5	27.0	5.5	0.5	1.5	2.0	2.3	2.3
783	39.0	25.5	5.0	0.5	1.4	1.9	2.1	2.1
784	41.8	28.0	5.7	0.6	1.6	2.0	2.3	2.2
792	38.0	24.8	5.0	0.5	1.6	2.5	2.5	2.2
795	39.4	25.0	5.4	0.4	1.5	2.5	2.5	2.3
798	37.0	24.0	5.0	0.5	1.6	2.2	2.5	2.4
805	42.5	27.8	5.4	0.5	1.5	2.0	2.1	2.1
806	39-5	26.2	4.9	0.4	1.5	8.1	2.2	2.2
verage	8	- •••	·	0.5	1.5	2.0	2.3	2.3

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TABLE 11-Series R

CAT.	BODY	TAIL LFNGTH	AMOUNT	3 DAY AMOUNT REG.	6 DAY AMOUNT REG.	9 DAY AMOUNT REG.	AMOUNT REG.	AMOUNT REG.	32 DAY AMOUNT REG.
703	39.4	25.5	9.6	0.6	2.0	2.8	3.8	4.5	4.5
701	41.0	26.8	9.8	0.6	, 2.2	2.9	3.8	4.9	4.9
721	40.0	25.4	10.0	0.6	2.0	3-3	4.2	4.4	4.8
731	37 - 5	24.0	10.0	0.6	2.0	3.0	4.0	5.1	5.1
722	39.4	25.2	10.0	0.6	2.0	3.1	4.1	5.0	5.0
732	37.3	23.4	10.0	0.6	2.0	3.0	3.9	4.9	4.9
745	38.8	25.3	9.6	0.6	1.9	2.8	4.I	4.4	4.6
758	41.0	27.5	9.8	0.7	1.9	2.8	4.0	4.0	4.1
762		26.0	10.5	0.7	2.1	3.8	4.3	5.0	5.0
767	40.4	26.0	10.0	0.7	2.3	3 - 4	4.0	4.9	4.9
768	40.5	26.2	10.0	0.7	2.3	3.8	4.1	5.4	5.4
778	41.4	26.6	11.0	0.6	2.3	3.6	4.9	5.5	5.5
780	39.2	25.0	9.6	0.7	1.9	2.9	4.0	5.0	5.0
786	40.4	25.7	10.5	0.6	2.1	3.2	4.1	4.3	4.3
796	40.5	26.0	10.0	0.7	2.0	3.0	3.8	4.9	4.9
803	38.5	24.5	10.0	0.7	2.0	3.2	4.I	4.3	4.3
804	40.4	26.6	11.0	0.6	2.3	3.7	4.4	5.2	5.2
808	39.0	24.5	10.2	0.6	2.3	3.7	4.5	5.2	5.2
807	42.0	27.8	9.8	0.6	1.9	3.0	4.0	5.0	5.0
834	41.5	26.0	10.0	0.6	2.1	3-4	3.8	4.1	4.2
Avera	ge		10.4	0.6	2.0	3.2	4.0	5.0	5.0

TABLE 12-Series S

		,							1	1
CAT.	BODY	TAIL LENGTH	AMOUNT REMOVED	3 DAY AMOUNT REG.	6 DAY AMOUNT REG.	9 DAY AMOUNT REG.	}	15 DAY AMOUNT REG.	1	32 DAY AMOUNT REG.
710	40.4	26.2	15.0	0	2.3	4.5	5.9	6.6	7.0	7.2
714	39.0	24.7	14.5		2.3	5.1	6.3	7.5	7.5	7.4
717	39.4	25.0	14.0	•	2.4	4.9	5.8	6.7	7.0	7.1
724	43.0	27.8	14.7	0	2.4	5.0	6.3	6.8	7.5	7.3
737	40.7	25.3	14.5	0	2.3	4.8	5.6	6.3	7.1	7.1
739	42.0	27.0	14.4	0	2.3	4.4	4.6	5.0	6.2	6.4
740	41.0	26.8	15.0	0	2.4	5.0	5.7	6.0	6.9	6.9
750	42.5	27.5	15.0	0	2.4	5.1	5.6	6.8	6.9	7.0
757	37.2	25.0	14.0	0	2.3	4.6	5.6	7.5	7.2	7.2
761	40.4	27.2	15.2	0	2.4	5.3	5.9	6.8	7.5	7.5
770	41.5	27.0	15.0	0	2.4	4.7	5.6	6.7	7.2	7.2
772	42.0	26.8	14.0	0	2.5	4.5	5.6	6.1	7.0	7.0
774	43.0	28.0	14.5	0	2.4	4.5	5.8	6.5	6.7	6.8
776		25.8	15.0	0	2.5	4.5	5.9	6.0	7.0	7.0
785	39.5	25.6	15.0	0	2.5	4.3	4.9	6.8	6.8	6.8
788	40.0	25.3	15.0	•	2.5	4.8	5.8	7.0	7.2	7.2
793	36.5	25.0	15.0	0	2.6	4.8	6.1	7.0	7.4	7.2
794	39.0	25.7	14.8	0	2.5	5.0	5.7	7.7	7.7	7.2
799	41.5	26.5	15.0	0	2.5	4.8	6.0	6.9	7.4	7.4
800	38.4	24.0	15.5	0	2.8	5.0	6.0	6.7	7-4	7.4
Averag	ge		14.8	0	2.4	4.9	5.8	6.7	7.2	7.2

TABLE 13-Experiment 4

	3 1	DAY	6	DAY	9	DAY	12	DAY	15	DAY	18	DAY	32	DAY	MOVED
SERIES	Amount	Per Cent	Amount	Per Cent	Amount	Per Cent	Amount	Fer Cent	Amount	Per Cent	Amount	Per Cent	Amount	Per Cent	AMOUNT RE
Series O Series P Series R Series S	0.5 0.6	13 10 6	1.0 1.5 2.0 2.4	31 29 20 16	I.4 2.0 3 2 4.9	44 40 31 33	1.4 2.3 4.0 5.8	44 44 40 39	1.4 2.3 5.0 6.7	44 44 48 45	1.4 2.3 5.0 7.2	44 44 48 48	I.4 2.3 5.0 7.2	44 44 48 48	3.2 5.2 10.4 14.8

TABLE 14—Series AA and BB
Series AA

NO.	BODY	TAIL LENGTH	AMOUNT REMOVED	2 DAY AMOUNT REG.	5 DAY AMOUNT REG.	7 DAY AMOUNT REG.	IO DAY AMOUNT REG.
	mm.	mm	mm.	mm.	mm.	mm.	mm.
900	19.0	11.0	2.3	0.8	1.3	i 1	
902	20.0	11.0	2.5	0.9	1.3		
904	19.0	11.0	2.5	0.8	1.2	1.3	1.3
906	18.5	11.0	2.8	0.9	1.4	1.5	1.5
908	21.0	12.0	3.0	0.8	1.3	1.4	1.5
910	18.0	10.5	2.5	0.8	1.2	1.3	1.3
912	20.0	12.0	2.8	0.9	1.4	1.5	1.5
914	18.0	10.5	3.0	0.9			
916	18.0	10.2	2.8	0.8	1.5	! !	1
918	18.0	10.5	2.7	0.8	1.5	1.5	1.5
920	18.6	11.0	2.8	0.8			i
922	18.0	10.5	2.6	0.8			1
verage			2.6	0.8	1.3	1.4	1.4

### Series BB

				. 5.6			2.8	
9	23	18.2	10.6	5.2	1.1	•		ı
9	21	19.0	11.0	5.7	1.2			1
9	19	18.2	11.0	5.5	1.1	2.4	2.8	2.8
9	17	18.5	10.6	5.4	1.3	2.4		
9	15	20.0	11.8	6.1	1.2			
9	13	18.2	10.5	5.5	1.2	2.5	2.6	2.6
9	II	18.2	11.2	6.2	1.1	2.6	3.1	3.4
9	109	19.0	12.0	5.8	1.2	2.5	2.5	2.8
9	107	19.5	12.0	6.2	1.2	2.6	3.2	3.5
9	05	19.0	11.0	5.2	1.1	2.4	2.5	2.6
9	03	19.0	10.5	5-4	1.2	2.5		
9	10	19.0	11.0	5.6	1.2	1.5		

TABLE 15-Series CC, Experiment 7

NO.	BODY	TAIL	AMOUNT	2 DAY	3 DAY	4 DAY	5 DAY	7 DAY	12 DA
	LENGTH	LENGTH	REMOVED	REG.	REG.	REG.	REG.	REG.	REG.
	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
036	29.2	19.0	5.5	0.7	1.4	8.1	2.3	2.8	2.8
039	28.2	16.2	6.0	0.7	1.6	1.9	2.3	2.9	2.9
040	26.5	17.0	5.0	0.8	1.5	1.8	1.8	2.4	2.4
043	31.2	21.0	5.2	0.8	1.6	1.8	2.3	2.7	2.7
044	31.0	19.0	6.0	0.7	1.6	1.9	2.2	2.6	2.6
047	30.0	19.2	5.0	0.7	1.4	1.8	2.1	2.6	2.6
049	27.5	17.3	5.0	0.7	1.5	1.9	2.3	2.6	2.6
051	26.1	16.0	5.0	0.7	1.5	1.9	2.2	2.9	2.9
053	28.0	17.5	5.0	0.7	1.5	1.8	2.2	2.4	2.4
verag	ges		5.3	0.7	1.5	1.8	2.2	2.7	2.7

There was measurable regeneration at the end of the first day (considered as 0.1 mm.)

TABLE 16-Series DD, Experiment 7

Body Length	TAIL LENGTH	AMOUNT REMOVED	2 DAY AMOUNT REG.	3 DAY AMOUNT REG.	AMOUNT REG.	5 DAY AMOUNT REG.	7 DAY AMOUNT REG.	AMOUNT REG.	I2 DAY AMOUNT REG.
mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
26.2	16.2	10.0	0.9	2.6	3.1	3.2.	4.2	5.0	5.0
32.0	20.2	10.0		2.6	3.2	3.5	4.3	4.9	4.9
27.2	17.2	10.0	1.0	2.4	3.3	3.9	4.3	5.0	5.c
32.0	21.0	10.2	0.1	2.5	3.2	3.8	4.4	4.8	4.9
29.0	18.0	10.0	1.0	2.6	3.2	3.6	4.1	5.0	5.0
28.2	17.0	9.8	1.1	2.5	3.3	3.6	4.4	5.1	5.1
29.0	18.0	10.0	1.1	2.5	3.4	3.7	4. I	5.2	5.2
27.0	17.0	9.9	0.9	2.3	3.1	3.4	4.3	4.9	4.9
31.0	19.2	9.8	0.9	2.5	3.3	3.7	4.1	5.3	5.3
Average		9.9	1.0	2.5	3.2	3.6	4.2	5.0	5.0

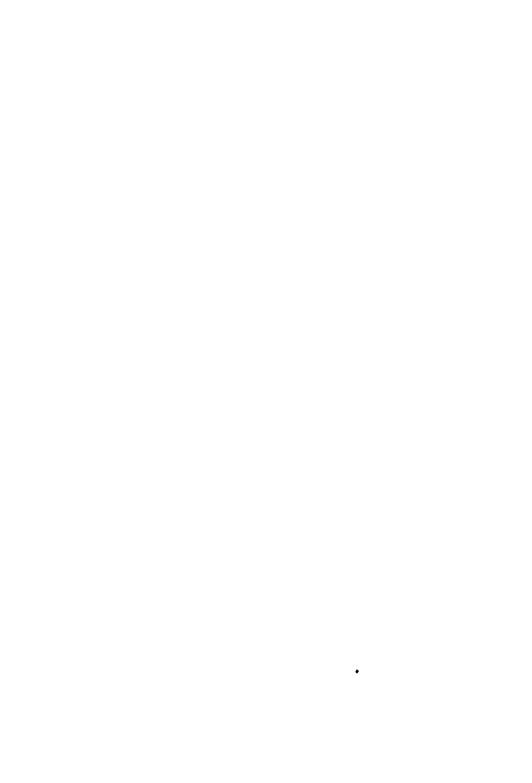
## Amount of Tail Regenerated to Removed in Tadpoles 455

TABLE 17-Averages Series CC and DD Experiment 7

AND THE PARTY OF T	}	I D	AY	2 D	AY	3	DAY	4 D	AY	5 1	DAY	7 1	DAY	10	DAY	12	DAY
SERIES	AMOUNT	Amount	Per Cent	Amount	Per Cent	Amount	Per Cent	Amount	Per Cent	Amount	Per Cent	Amount	Per Cent	Amount	Per Cent	Amount	Per Cent
Series CC Series DD	. 5.3		2	0.7			28		36		41				51		- 51

TABLE 18-Series EE and FF, Experiment 8

-	COVED	3 r	PAY	5 D	AY	6 р	Α¥	8 p	AY	12 1	DAY	15 I	AY	18 1	DAY	. 2I I	PAY
SERIES	AMOUNT REA	Amount	Per Cent	Amount	Per Cent	Amount	Per Cent	Amount	Per Cent	Amount	Per Cent	Amount	Per Cent	Amount	Per Cent	Amount	Per Cent
Series El Series Fl	, -	1 -1	25	1	33		36 33	mm. 2.3 3.6	45	mm. 2.3 4.0	45	mm. 2.3 4.3	45 43	mm. 2.3 4.4	}	mm. 2.3 4.4	45 44



## THE EFFECT OF ELECTRICAL STIMULATION UPON THE RATE OF REGENERATION IN RANA PIPIENS AND AMBLYSTOMA JEFFERSONIANUM<sup>1</sup>

### OREN E. FRAZEE

WITH TWO FIGURES

### INTRODUCTION

The object of the experiment was the determination of the effect of electrical stimulation upon the rate of regeneration. Larvæ of Rana pipiens and Amblystoma jeffersonianum were used. These forms were selected because of their abundance, and because of the work which has already been done upon their regeneration.

### **METHODS**

The general method consisted of the selection of a number of individuals divided into two equal sets,<sup>2</sup> one of which was to be stimulated, and the other not stimulated. An effort was made to have like conditions at all times for both the stimulated and the non-stimulated individuals. The food, light, temperature and handling are among the factors which were regarded as important to control. The factor of age is another important one in its bearing upon the rate of regeneration. For this reason, animals of the same age were selected.

The tadpoles were kept in small berry dishes. Cleanliness was emphasized and the dishes were cleaned thoroughly daily. Thin glass covers were placed over the dishes about 5 mm. above the

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<sup>\*</sup> See the various experiments for the modifications of the method of applying the current.

tops of the dishes. This minimized the amount of dust entering and still permitted a free circulation of the air.

Spirogyra was used as food for the tadpoles, and tubifex worms for the salamander larvæ. Stale food was not permitted. An excess of food rather than an undersupply was the rule.

No record has been kept of the temperature. It is known, however, to have been fairly constant. There have been no great fluctuations in the temperature during the time of an experiment.

An effort was made to handle the animals of the stimulated group in the same manner as the animals of the non-stimulated group, save for the stimulation which was given the former.

In the measurement of the entire body, tenths of millimeters were secured. In the measurement of the regenerated part, an ocular micrometer was used and it was possible to secure the measurements in hundredths of millimeters.

The cut was made in all cases as nearly as possible at the same level, at right angles to the notochord, and at the same time. A sharp razor was used for the cutting operation. The operation proceeded alternately with the stimulated and the non-stimulated ones.

The general methods of using the current were as follows:
(1) The current was taken from an electric lighting wire (alternating current), passed in series with an incandescent lamp, and connected up in series with a resistance coil. The tadpoles were placed in tap water in a wooden trough insulated on the inside with paraffine. They were screened from the electrodes as shown in Fig. 1. (2) The current was shunted from a resistance coil as shown in Fig. 2.

### DATA

Experiment 1. In this experiment 12 tadpoles of Rana pipiens were taken. They were of the same size; were operated upon October 9, 1908, when 10 mm. of the tail were removed. Three days later, the current from six dry battery cells was sent through each one of the tadpoles, the animal being in direct

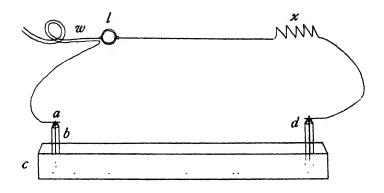


Fig. 1

### EXPLANATION OF FIG. 1

- c. Insulated wooden trough.
- b, Glass tube.
- a, Carbon electrode. (screened).
- d, Cork, held in neck of bottle and perforated for the carbon electrode.
- x, Resistance.
- 1, Incandescent amp.
- w, Line wire.

### EXPLANATION OF FIG. 2

- c, Insulated wooden trough.
- x, Resistance. .
- w, Line wire.
- k, Shunt.

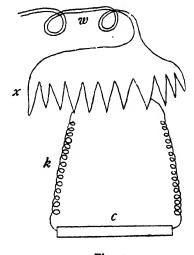


Fig. 2

contact with the terminals for ten seconds. Upon the following day this stimulation was repeated with the same six. Six days after the operation and three and two days after stimulations, measurements were taken which showed a slight advantage in the stimulated set. The following table shows amounts of regeneration in the six days for each tadpole:

TABLE I

Stimulated	Non-stimulated				
mm.	mm.				
1 0.40	7 o.40				
2 0.36	8 0.36				
3	9 0.36				
4 0.52	10 0.48				
5 0.52	11				
6 0.52	12 0.52				
die Plante					
Average 0.466+	Average 0.44+				

Experiment 2. In this experiment 20 tadpoles of Rana pipiens were taken. They ranged from 25 mm. to 28 mm. in length. They were operated upon October 22, 1908, when 10 mm. of the tail of each were removed. The following day 10 of the 20 were stimulated with the current from an electric lighting wire, voltage 110. The entire current was run through a small tank of water in which the tadpoles were placed for five minutes. None of the tadpoles survived the shock of the current. No measurement of the current was secured.

Experiment 3. In this experiment the 20 tadpoles of Rana pipiens which were selected, ranged from 29 to 35 mm. in length. November 16, 1908, 10 mm. of the tail of each were removed. The following day half of the tadpoles were charged with a current from the electric lighting wire, with a 16 c. p. lamp as resistance thrown in circuit, for ten minutes. Voltage, 110. The small tank of water mentioned in No. 2 and described above was used. At the end of ten minutes, 5 of the 10 had been killed and the remaining 5 died within a few hours after their removal to the dishes. No measurement of the current was secured.

Experiment 4. In this experiment 20 tadpoles of Rana pipiens were selected ranging from 39 to 47 mm. in length. They were operated upon December 1, 1908, when 10 mm. of the tail were removed. Ten were stimulated December 2, 3, 4, 7, 8, and 10, for 5 minutes each day. Current density 1660 8. Voltage, 110. Four of the stimulated and two of the non-stimulated ones died. A comparison of the regeneration of the remaining animals of the stimulated set and of the non-stimulated ones shows about 13 per cent greater amount of growth in the non-stimulated ones. The following table shows the amounts of regeneration together with the averages for each:

TABLE II						
Non-stimulated	mm.	Stimulated	mm.			
I	3.15	11	2.79			
2	Died	12	1.62			
3	2.18	13	2.43			
4	Died	14	2.25			
5	1.62	15	Died			
6	2.16	16	1.71			
7	2.97	17	1.71			
8	2.32	18	Died			
9	1.98	19	Died			
10	2.43	20	Died			
Average	2.35+	Average	2.08			

Experiment 5. In Experiment five 20 tadpoles of Rana pipiens were selected, ranging in length from 48 to 52 mm. They were operated upon December 7, 1908, at which time 8 to 12 mm. of the tail were removed. Ten of the 20 were stimulated December 8, 1908, for fifteen minutes. Current density 1660  $\delta$ . Voltage, 110. This was repeated upon the following day. All were measured December 15, 1908. The following table shows the amount of regeneration for each. The averages give an advantage of  $4\frac{1}{6}$  per cent to the non-stimulated set:

<sup>&</sup>lt;sup>3</sup> δ as proposed by Hermann and Matthias, is a unit of current density equal to one-millionth of an ampere of current per square millimeter. See C. B. Davenport, Experimental Morphology, part i, p. 128.

TABLE III

Non-stimulated	Stimulated
mm.	mm.
1 1.17	11 1.26
2 1.35	12 1.35
3 1.44	13 1.35
4 I.44	14 1.35
5 I.44	15 1.44
6 r.44	16 1.44
7 I.44	17 1.44
8 1.44	18 1.44
9 1.62	19 1.44
10 1.62	20 1.62
-	W Construction
Average 1 . 44	Average 1.38+

Experiment 6. Twenty larvæ of Amblystoma jeffersonianum ranging in length from 12 to 14 mm. were selected. All were of the same age, seven days. All were operated upon February 19, 1909. From 2 to 3 mm. of the tail were removed. Ten were stimulated February 23, 1909, in the following manner: Current on for three minutes; off one-half minute; on five minutes; off one minute; on five minutes; off one minute; on two minutes; then off, totaling fifteen minutes of current. The current density was 116.6  $\delta$ , the voltage 110. The amount regenerated was measured February 25, 1909, or two days after stimulation and six days after operation. The following table shows the amount of regeneration together with the averages for each larva. The table shows that the members of the non-stimulated set regenerated 10 per cent more than the stimulated ones:

TABLE IV

Non-:timulated	Stimulated
mm.	mm.
1 1.61	11 1.42
2 1.5	12 1.4
3····· 1.6	13 1.5
4····· 1.6	14 1.1
5 1.6	15 1.42
6 I.41	16 1.5
7····· 1.45	17 1.41
8 1.5	18 1.45
9 1.55	19 1.38
10	20}
Average 1.53+	Average 1.20+

Experiment 7. For this experiment 80 larvæ of Amblystoma jeffersonianum were selected. They were measured and operated upon March 12, 1909. Forty were stimulated March 15, 1909, for six minutes. Current density 10 δ. Voltage 110. Measurements of the amount regenerated were taken March 17, 19, 21, and 23, 1909.

Those numbered 1-5, 11-16, 21-26, etc., are individuals of the non-stimulated set; those numbered 6-10, 16-20, 26-30, etc., are members of the stimulated set.

Tables V, VI and VII give all the measurements taken during the experiment.

An examination of these measurements shows:

- I That two days after the stimulation each individual of the stimulated set had regenerated on the average 0.044 + mm., or nearly 5 per cent more than the members of the non-stimulated set. See Tables V and VI, measurement number one.
- 2 That four days after the stimulation each individual of the stimulated set had regenerated on the average 0.063 + mm., or nearly 4 per cent more than the members of the non-stimulated set. See Tables V and VI, measurement number two.
- 3 That six days after the stimulation each individual of the stimulated set had regenerated on the average 0.06 + mm., or 2.6 + per cent more than the members of the non-stimulated set. See Tables V and VI, measurement number three.
- 4 That eight days after the stimulation each individual of the stimulated set had regenerated on the average 0.086 + mm., or nearly 2\frac{3}{4} per cent more than the members of the non-stimulated set. See Tables V and VI, measurement number four.
- 5 That 40 days after the beginning of the operation, each individual of the stimulated set had a greater total length on the average of 1.036 + mm., or 10 + per cent more than the individuals of the non-stimulated set, although their average total lengths at the beginning were the same. This shows that the stimulation increased the rate of general growth of the animal as well as its rate of regeneration. See Table VII.

Experiment 8. This experiment included 34 larvæ of Amblystoma jeffersonianum ranging from 23.5 to 31 mm. in length.

<sup>4</sup> See measurements, Tables V. VI, and Table VII.

TABLE V Stimulated Larvæ

No.	ENTIRE LENGTH OF LARVA	LENGTH OF BODY ALONE	LENGTH OF TAIL	AMOUNT REMOVED	FIRST MEASURE- MENTS OF REGENER- ATED LENGTHS	SECOND MEASURE- MENTS	THIRD MEASURE- MENTS	FOURTH MEASURE- MENTS	LENGTH OF LARVA 40 DAYS AFTER EX PERIMENT BEGUN
6	26	13	13	7	0.6	1.5	2	3.1	34
7	*					-			•
8	25	13	12	. 7	0.9	1.75	2	3.1	38
9	26.5	14.5	12	. 8	0.9	1.8	2.5	3.7	0
10	23.5	12.5	11	6	0.8	1.4	2.1	2.95	•
16	24	12.5	11.5	7	0.9	1.7	2.4	3.7	•
17	25.5	13	12.5	7.5	0.9	1.65	2.25	3.3	33.5
18	*	'		! !		1			0
19	28.5	14.5	14	9	1.2	1.95	2.65	?	36.5
20	23	12.5	10.5	7	1	1.6	2.35	3.71	31
26	23	11	12	8	0.7?	1.2	2.15	2.9	37
27	21	11	10	6	0.89	1.65	2.45	3.6	31.5
28	23	12	11	7	o 89	1.55	2	3	40
29	25.5	13	12.5	7 · 5	09	1.7	2.6	3.7	40
30	25	13	12	8	0.9	1.81	2.4	3.5	36
36	24.5	13	11.5	7.5	0.95	1.6	2.1	3.2	34.5
37	25.5	14	11.5	7	1.1	2.1	2.65	3.71	35.5
38	26	14	12	7	1.2	2.1	2.7	3.7	0
39	25	13	12	7	1	2	2.55	٠	32.5
40	24.5	13	11.5	7.5	0.84	1.5	1.87	٩	35.5
46	23	12	11	6.5	0.7	1.3	1.9	3	0
47	23.5	12	11.5	7	0.85	1.55	2.1	3.1	38.5
48	25	13.5	11.5	8	0.7	D	İ	,	0
49	25	13	12	6	0.75	1.5	2.1	2.95	0
50	26	14	12	7	0.8	1.5	1.75	2.8	39
56	25	13	11.5	7	0.85	1.65	2.2	3.1	37
57	25 5	13	12.5	7	0.85	1.65	2 I	3.2	0
58	28	14.5	13.5	7	1	1.85	2.6	3	33
59	26	13	13	7.5	0.95	1.75	2.35	3 · 7	33 · 5
60	25 *	13.5	11.5	7	0.8	1.6	2.3	3.6	32.5
66		į				_	[	' I	0
67	23	12	11	6	0.997	1.6	2.0	3	37
68	26.5	13 5	13	6.5	0.91	1.92	2.6	3.9	35
69	25 5	13	12.5	7	0.98	1.65	2.25	3.51	0
70	24	12	12	8	0.8	1.56	2.3	3.2	36
76	26	14	12	7.5	1.1	1.95	2.8	3.85	37
77	21	11	10	7	1.5	2	2.9	, ?	0
78	22	11.5	10.5	6.5	0.92	1.8	2.65	٦	34.5
79 80	25 26	13	12	6.25	1.5	2	2.8	3.7	38.5
60	20	13	13	8	0.85	1.51	2.3	3.2	38.2

Non-stimulated Larva

NO.	ENTIRE LENGTH OF LARVA	LENGTH OF BODY ALONE	;	AMOUNT REMOVED	FIRST MEASURE MENTS OF REGENER- ATED LENGTHS	SECOND MFASURE- MENTS	1	FOURTH MEASURE- MENTS	LENGTH OF LARVA 40 DAYS AFTER EX- PERIMENT BEGUN
·1	24.5	13	11.5	7	0.75	1.2	1.8	2.8	0
2	26	:14	12	6	0.75	1.35	2.5	2.8	0
3	23.5	13	10.5	6.5	0.8	1.4	2	3.0	0
4	22	11	10	7	0.7	1.4	2	2.8	31
5	*			,		;			0
11	25	13	12	6.5	0.9	1.6	2.1	3	0
12	27.5	14	13.5		I	1.85	2.4	3.55	35
13	22.5	12	10.5	7	1	1.5	2.2	3.2	31
14	25	12.5	12.5	8	0.8	1.7	2.2	3.5	34.5
15	26	14	12	8 .	I	1.65	2.35	3.7	36
21	25.5	14	11.5	7.5	0.9	1.55	2.25	3.1	36
22	25.5	13	12.5	8	1.1	1.8	2.4	3.5	33
23	*	_	1			1	. !	• •	0
24	25	13	12	7.5	0.65	1.2	1.95	2.9	31
25	21	11	10	5.5	0.7	1 7	1.47	. ₹	•
31	27.5	14	13.5	8	0.9	njenje			35
32	25	13	12	7	1	1.8	2.65	?	36.5
33	26.5	14 ;	12.5		0.95	1.9	2.75	?	36.5
34	24	13	11	7	1 ;	1.55	2	3	34.5
35	23.5	11.5	11	6.5	1 1	1.75	2 ?	ا د	34
4I	25	12.5	12.5	7	0.99	1.9	2.6	3.75	33
42	25	13	12	7	0.89	1.6	2	3.1	35
43	25	13.5	11.5	7	0.77	1.5	2	3.1	35
44	25	13	12	6.5	0.89	1.6	2.3	3.5	33.5
45	28	14.5	13.5	7 :	0.9	1.9	2.4	3.4	37.5
51	26	13.5	12.5	8 1	0.7	1.62	2.1	2.85	36
52	24	12	12	7	0.8	1.62	2	3	33.5
53	24	13	11	6.5	0.85	D	-	3	0
54	24	13	11	7	0.8	1.6	2.4	3.3	45
55	25.5	13.5	12	7	0.85	1.62	2.2	3.1	34
61	24.5	13	11.5	7	0.9	1.7	2.5	3.6	33
62	23.5	12.5	11	5.5	0.9	1.55	2	3.1	34.5
63	22	11.5	10.5	5.5	0.85	1.7	2.4	3.2	35
54	26	13	13	6.5	0.75	1.5	1.77	7	35 35
65	26	13	13	7	0.89	1.65	2.3	3.4	33 38
71	20	10.5	9.5	6	0.71	1.6	2.2	3.5	0
72	25	13.5	11.5	7	1.1	1.8	2.7	3.8	•
73	25.5	14	11.5	6	1.1	1.85	2.5	3.2	0
4	22	11	11	7	1.0	1.7	2.6	3.7	35.5
5	25	13.5	11.5	7	1.1	1.8	2.65	3.1	33.3

TABLE VI

Table showing the four measurements of regenerated lengths in both sets, arranged in order of amount together with totals and averages.

M	ı	м	2	M	1	м	4
Non-stim.	Stim.	Non-stim.	Stim.	Non-stim.	Stim.	Non-stim.	Stim.
0.65	0.6	1.2	1.2	1.8	1.9	2.8	2.8
0.7	0.7	I.2	1.3	1.95	1.95	2.8	2.9
0.7	0.7	1.35	1.4	2	2	2.8	2.95
0.75	0.75	1.4	1.5	2	2	2.95	2.95
0.75	0.8	1.4	1.5	2	2	2.9	3
0.75	0.8	1.5	1.5	2	2	3	3
0.77	0.8	1.5	1.5	2	2.1	3	3
0.8	0.8	1.5	1.51	2	2.1	3	3.1
0.8	0.84	1.55	1.55	2	2.1	3	3.1
0.8	0.85	1.55	1.55	2.1	2.I	3.1	3.1
0.8	0.85	1.55	1.56	2.1	2.1	3.1	3.1
0.85	0.85	1.6	1.6	2.2	2.15	3.1	3.2
0.85	0.85	1.6	1.6	2.2	2.2	3.1	3.2
0.85	0 89	1.6	1.6	2.2	2.25	3.1	3.2
0.89	0.89	1.6	1.6	2.2	2.25	3.2	3.2
0.89	0.9	1.6	1.65	2.25	2.3	3.2	3.3
0.89	0.9	1.62	1.65	2.3	2.3	3.2	3.5
0.9	0.9	1.62	1.65	2.3	2.3	3.3	3.51
0.9	0.9	1.62	1.65	2.35	2.35	3.4	3.6
0.9	0.9	1.65	1.65	2.4	2.35	3.4	3.6
0.9	0.9	1.65	1.7	2.4	2.4	3.5	3.7
0.9	0.91	1.7	1.7	2.4	2.4	3.5	3.7
0.9	0.92	1.7	1.75	2.4	2.45	3.5	3.7
0.95	0.95	1.7	1.75	2.4	2.5	3.5	3.7
0.99	0.95	1.7	1.8	2.5	2.55	3.55	3.7
1.0	0.98	1.75	1.8	2.5	2.6	3.6	3.7
1.0	1.0	1.8	1.81	2.5	2.6	3.7	3.71
1.0	1.0	1.8	z.85	2.6	2.6	3.7	3.71
1.0	1.1	1.8	1.92	2.6	2.65	3.75	3.85
1.0	1.1	1.8	1.95	2.65	2.65	3.8	3.9
1.0	1.2	1.85	1.95	2.65	2.65	3.9	,
1.0	1.2	1.85	2	2.7	2.7	1 '	
1.1	1.2	1.9	2	2.75	2.8	<i></i>	
1.1	1.5	1.9	2		2.8		
1.1	1.5	1.9	2.1		2.9		
1.1			2.1			•	
32.23	32.88	57.01	60.90	75.40	82.05	101.35	100.68
v895+	.939+	1.628+	1.691+	2.284+	0.2344+	3.27-	3.356

TABLE VII

Showing: (1) Original total lengths; (2) Total lengths 40 days later

RIGINAL TOT	AL LENGTHS	LENGTHS 40	DAYS LA
Non-stim.	Stim.	Non-stim.	Stim.
31	34	24.5	26
35	38	26	25
31	33.5	23.5	26.5
34.5	36.5	22	23.5
36	31	25	24
36	37	27.5	25.5
33	31.5	22.5	28.5
31	40	25	23
35	40	26	23
36.5	36	25.5	21
36.5	34.5	25.5	23
34.5	35.5	25	25.5
34	32.5	21	25
33 :	35.5	27.5	24.5
35	38.5	25	25.5
35	39	26.5	26
33.5	37	24	25
37.5	33	23.5	24.5
36	33.5	25	23
33.5	32.5	25	23.5
34.5	37	25	25
34	35	25	25
33	36	28	26
34.5	37	26	24.5
35	34.5	24	25.5
35	38.5	24	28
38	38.5	24	26
35.5	i	25.5	25
37		24.5	23
į		23.5	26.5
		22	25.5
1	į	26	24
		26	26
1		20	21
1		25	22
		25.5	25
		22	26
		25	
34.586	35.722	24.6+	24.7

EXPLANATIONS OF ABBREVIATIONS IN TABLE V, VI AND VII

<sup>\*</sup> Died before operation.

D Died sometime after operation, while experiment was still in progress.

<sup>?</sup> Not sure of measurement.

<sup>\*</sup> Tail slightly mutilated.

They were measured May 6, 1909, and operated upon the following day, when from 5.5 to 8.5 mm., of the tail were removed. Upon the same day, i. e., day of operation, 17 were stimulated for one hour. Current density, 8.3  $\delta$ . Voltage 110.

Six larvæ did not survive the shock of the cutting and current, and died within an hour after the stimulation. The remaining ones of the stimulated set were much depressed, would not eat for several hours, and were not easily disturbed. Three days after stimulation, they appeared shrunken as compared with the ones in the non-stimulated set. They were at this time, however, just as active apparently as the ones in the non-stimulated set. Regenerated tissue, under the microscope looked alike in both sets. Five days after the stimulation the stimulated individuals looked and behaved in a manner quite similar to the non-stimulated individuals.

Tables VIII and IX show the various measurements taken. In general the advantage is in favor of the non-stimulated set. It is noticeable, however, that the stimulated ones hold their own, and with the return of bodily vigor the rate of regeneration increases.

An examination of these measurements shows:

- I That three days after stimulation the individuals of the nonstimulated set had regenerated on the average 0.03 + mm., 7½ per cent more than the individuals of the stimulated set. See Tables VIII and IX, measurement number one.
- 2 That five days after stimulation the individuals of the nonstimulated set had regenerated on the average 0.09 + mm., or 8 + per cent more than the individuals of the stimulated set. See Tables VIII and IX, measurement number two.
- 3 That seven days after stimulation the individuals of the nonstimulated set had regenerated on the average 0.06 + mm., or 2\frac{3}{4} per cent more than the individuals of the stimulated set. See Tables VIII and IX, measurement number three.
- 4 That nine days after stimulation the individuals of the nonstimulated set had regenerated on the average 0.035 mm., or I + per cent more than the individuals of the stimulated set. See Tables VIII and IX, measurement number four.

TABLE VIII Stimulated Larvæ

NO.	ENTIRE	LENGTH OF BODY	LENGTH OF TAIL	LENGTH	UREMENT OF REGEN- ERATED LENGTH MAY 10	SECOND MEASURE- MENT MAY 12	THIRD MEASURE- MENT MAY 14	FOURTH MEASURE MENT MAY 16
1*	30	16	, 14	7.7				
2*	28	15.5	12.5	7.5				
3*	. 27	15	12	7.3				
4*	30.5	16.5	14	7.4				
5*	27	15	12	7.3				
11*	26	15	1 11	8				
12	30	16	14	10	0 42	10	2.0	2.55
13	27	15	12	7.5	0 4	0.1	1.95	2.5
14	25.5	14.5	11	6.7	0.4	13	2.55	36
15	30	16	14	7.5	0.35	1.0	1.85	2.9
21	29	16	13	7 - 5	0.45	1.3	2.3	2.25
22	23.5	13 5	10	7.1	0.49	1.35	2.45	3 - 4
23	27.5	15	12.5	6.9	0.49	1.25	2.5	3.6
24	28.5	16.5	12	7	0.35	1.1	1.95	3.1
25	27.5	15	12.5	7.4	0.3	0.75	1.7	2.5
31	28	16	12	8	0.3	1.1	2.1	3.2
32	30	16.5	13.5	8	0.35	0.75	1.6	2.5

\*Died May 7, 1909, due to shock of operation and current.

## Non-stimulated Larvæ

				•••••				
				• -				
6	29	15.5	13.5	7.1	0.39	1.35	2.3	3.1
7	27.5	15	12.5	7	0.45	0.95	1.65	2.55
8	26.5	15	11.5	7.1	0.33	0.7	1.8	2.6
9	30 5	16.5	14	8.6	0.39	1.3	2.1	2.95
10	29	16	13	7 · 3	0.49	1.3	2.5	2.7
16	29	17.5	11.5	5.5	0.35	0.9	1.9	2.6
17	26	14 .	12	63	0.47	1.0	2	3.2
18	29	16.5	12.5	6?	0.39	1.1	1.7	2.5
19	25.5	15	10.5	6.8	0.4	1.3	2.2	3.1
20	27	16	11	6.8	0.42	1.4	2.5	3.5
26	23.5	13.5	10	7	0.48	1.25	2.1	3 - 3
27	27	15	12	7-4	0.48	1.3	2.35	3.1
28	27.5	15.5	12	7.2	0.4	0.95	1.9	3.2
29	• 27	15	12	7.4	0.4	1.3	2.3	3.3
30	31	16	15	8.5	0.4	1.45	2.8	3.5
33	27.5	15	12.5	6.7	0.4	1.0	2.1	3
34	28	16	12	8.5	0.5	1.35	2.2	3.5
	1		1		!			

Experiment 9. In this experiment 36 tadpoles of Rana pipiens ranging from 14 to 18.1 mm. in length were selected. They were measured and operated upon June 1, 1909, at which time 4.5 to 7 mm. of the tail were removed. The following day 18 were stimulated for one hour. Current density 5 &. Voltage, 40. They were measured two and four days after stimulation.

TABLE IX

Table showing the four measurements of regenerated tails in both sets, arranged in order of amounts, together with the totals and averages

M <sup>1</sup> MAY IO		M <sup>2</sup> MAY 12		, M <sup>3</sup> MAY 14		M <sup>4</sup> MAY 16	
Non-stim.	Stim.	Non-stim.	Stim.	Non-stim	Stim.	Non-stim.	Stim.
o 33	0.3	0.7	0.75	1.65	1.6	2.5	2.5
0.35	0.3	0.9	0.75	1.7	1.7	2.55	2.5
0.39	0.35	0.95	1.0	1.8	1.85	2.6	2.5
0.39	0.35	0.95	1.0	1.9	1.95	2.6	2.55
0.39	0.35	1.0	1.0	1.9	1.95	2.7	2.9
0.4	0.4	1.0	1.1	1 2 .	2	2.95	3.1
0.4	0.4	1.1	1.1	2.1	2.1	3	3.2
0.4	0.42	1.25	1.25	2.1	2 3	3.1	3.25
0.4	0.45	1.3	1.3	2.1	2.45	3.1	3.4
0.4	0.49	1.3	1.3	2.2	2.5	3.1	3.6
0.42	0.49	1.3	1.35	2.2	2.55	3.2	3.6
0.45		1.3		2.3		3.2	
0.47		1.3		2.3		3.3	
0.48		1.35		2.35		3.3	
0.48		1.35		2.5		3.5	
0.49		1.4		2.5		3.5	
0.5		1.45		2.8		3.5	
7-14	4.30	19.60	11.90	36.40	22.95	51.70	33.10
Av.o.42 :	39+	1.17+	1.08 +	21.4+	2.08+	3.04+	3.009-

The stimulated ones at no stage seemed to show any indication of depression, but on the contrary they appeared just as vigorous and plump as those of the non-stimulated set.

Tables X and XI show the various measurements taken. A distinct advantage is shown in favor of the stimulated set.

TABLE X
Stimulated Larva.

NO.	ENTIRE LENGTH	BODY LENGTH	LENGTH OF TAIL	REMOVED	MEASUREMENT NUMBER ONE	MEASUREMENT NUMBER TWO
1	18.1	7.1	11	5.5	0.5	1.5
2	16.5	6.5	10	5.5	0.4	1.1
3	15.5	6.5	9	, 6	0.45	1.2
4	17	8.5	9.5	5.7	0.51	1.6
5	16	7	9	5.5	0.45	1.2
6	15	6	9	5	0.38	1.3
7	15.6	6.6	9	5	0.4	1.3
8	17	7	• 10	5.5	0.4	1.6
9	16.5	7.5	9	6	0.4	1.5
10	14	5.5	8.5	4-5	0.4	1.5
11	16	7.5	8.5	5	0.45	1.5
12	15.5	7.5	8	5.5	0.45	1.6
13	15	6	9	5	0.4	1.4
14	16.5	6.5	10	6.5	0.45	1.5
15	16	7	9	5	0.42	1.4
16	14	5	9	5.5	0.45	1.6
17	16	6	10	5.5	0.5	1.6
18	14	5.5	8.5	5	0.45	1.5

Non-	 med	7 4	

19	15.5	6.5	9	5	0.38	1.2
20	16	7	9	5	0.25	1
21	16	7	9	5-5	0.25	1.3
22	16.5	7	9.5	6	0.35	1.4
23	15	6	9	5.2	0.4	1.3
24	16	7	9	5.5	0.3	1.3
25	15	7.5	8.5	5.5	0.3	1.4
26	15	7	8	4.5	0.25	1
27	16	7.5	8.5	5 6	0.4	1.4
28	17	7	10	6	0.3	1.4
29	16	7 6	9	5.5	0.4	1.4
30	15	6	9	5	0.4	1.5
31	17	7	. 10	7	0.3	1.3
32	17	7	10	6	0.35	1.4
33	17	9	8	6	0.35	1.2
34	16	9 8	8	6	0.35	1.4
35	16	7	9	6	0.4	1.3
36	18	7.5	10.5	7	0.4	1.4

TABLE XI

Table showing two measurements of regenerated lengths in both sets, arranged in order of amount and the totals and averages

M <sup>1</sup>		M <sup>2</sup>				
Non-stim.	Stim.	Non-stim.	Stim.			
0.25	0.38	1.0	1.1			
0.25	0.4	1.0	1.2			
0.25	0.4	I.2	1.2			
0.3	0.4	1.2	1.3			
0.3	0.4	1.3	1.3			
0.3	0 4	1.3	1.4			
0.3	0.4	1.3	1.4			
0.35	0.42	1.3	1.5			
0.35	0.45	1.3	1.5			
0.35	0.45	1.4	1.5			
0.35	0.45	1.4	1.5			
0.38	0.45	1.4	٠.5			
0.4	0.45	1.4	1.5			
0.4	0.45	1.4	1.6			
0.4	0.45	1.4	1.6			
0.4	0.5	1.4	1.6			
0.4	0.5	1.4	1.6			
	0.51	1.5	1.6			
6.13	7.86	23.6	25.9			
v34+	.43+	1.31+	1.43+			

An examination of these results shows:

- I That two days after stimulation each individual of the stimulated set had regenerated on the average 0.09 mm., or 26 + per cent more than the members of the non-stimulated set. See Tables X and XI, measurement number one.
- 2 That four days after stimulation each individual of the stimulated set had regenerated on the average 0.12 + mm., or 9 + per cent more than the members of the non-stimulated set. See Tables X and XI, measurement number two.

#### DISCUSSION

That growth in plants is accelerated by the electrical current within certain limits has been shown by a number of investigators. In the case of animals, Lombardini ('68) and Windle ('93, '95) found that a current passing transversely through the chick embryo caused it to develop abnormally, or to cease developing altogether. Rusconi ('40) believed that a slight current accelerated the development of the frog's egg. It is to be noted that very little work has been done to show the effect upon the growth of animal tissue, and so far as the author knows the present work is the first attempt to determine the effect of electricity upon regeneration.

The experiments here recorded indicate that an electrical stimulation of animal tissue may be beneficial for purposes of regeneration under two conditions: First, when the current density is small and the voltage comparatively low. Second, when the current density is small and the voltage comparatively high, provided that the stimulation be of short duration; furthermore, they show that any considerable degree of current density, or a high voltage applied for more than a very short time, decreases the rate of regeneration.

The data in Experiments 2 and 3,5 both of which were preliminary in their nature, show that a strong current is harmful. It shows in Experiments 4 and 5 with a current density of 1660  $\delta$  in each instance, and with a voltage of 110 that repeated daily stimulations with such a current are harmful whether the number of repetitions is two or six. In Experiment 6 with young individuals seven days old, repeated stimulations, at short intervals of time, with a current density of 116.6  $\delta$ , and voltage 110, is harmful, even if the total stimulation amounts to no more than fifteen minutes. In Experiment 7, with much older individuals, thirty days old, the data show that with a current density of 10  $\delta$  and voltage 110, a six-minute stimulation gives beneficial results. In Experiment 8 it is seen that with tadpoles of the same

<sup>&</sup>lt;sup>5</sup> In Experiment 1 the number of individuals taken for comparison is too small to make the result of much value.

age as in number seven, harmful results appear when the individuals are stimulated with a current density of 8.3  $\delta$  and voltage 110 for a period of one hour. In this set, however, the later behavior points toward positive effects. In number nine in which the individuals are slightly younger (and in which stimulation lasted for an hour), beneficial results occur with a current density of 5  $\delta$  and a voltage of 40. It is interesting to note, in connection with the effect of electrical stimulation upon the rate of regeneration, that slight stimulation seems to increase the animal's activity, while any considerable degree of stimulation decreases its activity.

#### SUMMARY

- I With a current density of 5  $\delta$  and a voltage of 40, stimulation of 18 of 36 individuals of Rana pipiens, 14 to 18.1 mm. in length, for an hour gave for the 18 stimulated ones, from 9 to 26 per cent more in the amount of tissue regenerated than for the remaining 18 non-stimulated.
- 2. With a current density of 8.3  $\delta$  and voltage of 110, stimulation of 17 of 34 individuals of Amblystoma jeffersonianum, 23.5 to 31 mm. in length, gave for the 17 stimulated ones from 1 to 8 + per cent less in the amount of tissue regenerated than for the remaining 17 non-stimulated.
- 3 With a current density of 10  $\delta$  and voltage of 110, stimulation of 40 of 80 individuals of Amblystoma jeffersonianum, from 20 to 28.5 mm. in length, gave for the 40 stimulated ones from 2.6 to 5 per cent more in the amount of tissue regenerated than for the remaining 40 non-stimulated.
- 4 With a current density of 116.6  $\delta$  and voltage 110, repeated stimulations at intervals of one-half minute to two minutes of 10 of 20 larvæ of Amblystoma jeffersonianum, from 12 to 14 mm. in length, gave for the 10 stimulated ones about 10 per cent less in the amount regenerated than the remaining 10 non-stimulated.
- 5 With a current density of 1660  $\delta$  and voltage of 110, repeated daily stimulations, of two or more times, of 10 of 20 individuals of Rana pipiens, from 39-52 mm. in length, gave for the 10 stimu-

lated ones  $6\frac{1}{4}$  to 13 per cent less in the amount regenerated than for the remaining 10 non-stimulated.

- 6 With a current density greater than given above decidedly harmful results appear.
- 7 With small current density and comparatively low voltage, stimulation of the tadpoles increased the rate of regeneration.
- 8 With small current density and a comparatively high voltage, stimulation of the tadpoles for a brief period increased the rate of regeneration.
- 9 With any considerable degree of current density, or with a high voltage applied for more than a very short time, stimulation of the tadpoles decreases the rate of regeneration.
- 10 With a current density of 10  $\delta$ , voltage 110, stimulation of 40 individuals showed that their average total lengths, forty days after the operation, were more than the average total lengths of the 40 non-stimulated.

#### ACKNOWLEDGMENT

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# THE EFFECT OF SUCCESSIVE REMOVAL UPON . THE RATE OF REGENERATION<sup>1</sup>

RY

#### CHARLES ZELENY

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#### I INTRODUCTION

The present paper contains the results of an analytical, quantitative study of the effect of successive removal of organs upon their rate of regeneration. It is one of a series of similar studies dealing with the effects of various external and internal factors. Because of the necessity of eliminating disturbing conditions in the case of experimentation with regard to any one factor, it was found desirable to make an extended simultaneous study of as many of the factors as possible. The present paper deals merely with successive regenerations. It was found in the best controlled series of experiments that successive removal causes either no change or an increase in rate of regeneration and not a decrease in rate.

<sup>&</sup>lt;sup>1</sup> Contributions from the Zoölogical Laboratory of Indiana University. No. 108.

In a determination of the rates of successive regenerations it is necessary to bear in mind the essential difference between two points of view. On the one hand the observer may be concerned merely with the gross result of the second and later removals as compared with the first without regard to the essential factor involved. He will then merely determine whether, for instance, a salamander's tail is replaced more slowly or more quickly the second time than the first. Even with this limited object in view, he is likely to get contradictory results in different sets of experiments, the results varying with the conditions.

On the other hand, he may seek to determine the effect of the successive removal itself without special regard to the gross character and amount of the successive replacements. The difficulty of such a determination is apparent and only an approximate value can be obtained. Most of the important sources of error may however be reached. Former results along this line are for the most part unreliable, because the co-ordinate accessory factors are neglected. For instance successive removals of an organ in an individual may show a change in rate of replacement when as a matter of fact the change is due not to the successive removals but to an age factor quite apart from removal. Or the experiment may reveal no change in rate, because the distinctly appreciable effect of successive removal is destroyed by the influence of accessory factors.

#### II GENERAL METHOD

The present paper is concerned as far as possible with the effect of successive removal alone. Some of the sets of experiments were, however, controlled with much more effectiveness than others. The principal disturbing factors that have been considered are the following:

- 1 Age.
- 2 Periodic physiological changes such as those due to the molting process in the Crustacea.
- 3 Physiological changes as a result of prolonged subjection to laboratory conditions.

- 4 Changes in rate during the regeneration period.
- Differences in the level of the call 6 Additional injury to the individual.
- Changes in temperature
- Changes in character and quantity of the food.
- Individual variation in rate of regeneration due to causes not otherwise controlled.

Most of these factors have been made the subjects of separate study, but we are concerned here merely with their elimination. It should, however, be stated that these studies have proved conclusively the necessity of disregarding data on successive regeneration from experiments that are not carefully controlled. This statement is made with a full realization of the obvious gaps in the present data, especially in some of the series. These gaps are emphasized in the discussions of the individual methods. A statement of the principal ways by which the chief disturbing factors were removed follows. The more special treatment of the various devices is reserved for the individual sets of experiments.

- The age factor was eliminated by means of several devices. The two principal ones are given here: (a) Whenever possible individuals of a single brood raised under identical conditions were used. Comparisons of later regenerations with the first were not made in the case of the successive regenerations of a single individual, but between two sets of individuals operated upon at the same time, one set for the later regeneration and the other for the first regeneration. (b) In two of the experiments the first regeneration of one organ or part of the body was compared with the second regeneration of a similar organ or part of the same individ-This device is of value only in determining the local effect of successive removal since it can show no difference between the two rates in case the effect is wholly constitutional.2
- 2 In Crustacea the periodic physiological changes due to the molting habit constitute the most formidable of the sources of error. A detailed account of the manner in which their elimination was undertaken is given under the separate experiments (pp.

Spallanzani (1769) recognized the importance of the age factor.

- 493, 494, 498, 499, 502). In every instance interval after the molt, since the neglect of this precaution has been found to be an important source of error.
- 3 It is a notorious fact that under prolonged subjection to laboratory conditions many animals gradually lose their vigor. This has been demonstrated for a large number of activities. It often merely indicates bad living conditions in the laboratory. In other cases, however, it is undoubtedly due to change of environment alone. At any rate the effect is one that cannot be neglected and seems always to be present. In the experiments on successive regenerations, comparisons were made only between individuals having the same laboratory histories.
- 4 A regenerating organ does not grow at a uniform rate from the time of the operation until the completion of the process. Two sets of individuals must therefore be compared only during corresponding periods. Miss Durbin<sup>5</sup> has studied in detail the change in rate following the removal of the tail of a frog tadpole and finds that the rate is low for a short period, then rises very rapidly to a maximum at from six to nine days, then sinks rapidly to a lower level and finally gradually approaches zero. The rapidity of the change shows the absolute necessity of the use of identical periods in a comparison.
- 5 The level of the cut must be the same in all operations of any two compared sets of individuals, because the rate varies with the level of the cut. Ellis has shown that in the tail of the frog tadpole the length regenerated after several days varies directly as the length removed. Care was taken to avoid this source of error in dealing with successive removals.
- 6 Since the rate of regeneration varies with the temperature, it is necessary to have the compared sets of animals as nearly alike in this regard as possible. By alternating the individuals of

<sup>3</sup> See especially Emmel '06 and Zeleny '05b.

<sup>.</sup> See Parker '03.

<sup>5</sup> See Durbin '09.

See Ellis '09.

<sup>7</sup> See especially Morgan '06 and Stockard '08.

two compared sets in their positions on the laboratory tables this source of error was eliminated. Preliminary experiments by Ellis ('09) indicate that the regeneration rate is even more susceptible to temperature changes than is the rate of ordinary growth.\*

- 7 Morgan ('06) has shown that in the salamander, Diemyctylus viridescens, the rate of regeneration in length of the tail is independent to a large degree of the food supply. A similar statement is made by Spallanzani (1769). That there is some influence is, however, shown by the observations of Miss Durbin on the effect of starving or change in food upon the rate of regeneration of frog tadpoles. In order to avoid any error due to this factor, all animals in an experiment were fed as nearly the same quantity and quality of food as possible.
- 8 It has been shown that the rate of regeneration of an organ in many cases is influenced by injuries to other parts of the same individual.<sup>11</sup> In the present experiments animals accidentally injured in any part of the body are discarded.
- 9 Experience with a great many sets of individuals leads me to believe that so-called individual variations in rate of regeneration are due not so much to inherent differences in the animals as to differences in the environment. Close attention to the minor details of the operation and care of the animals with special reference to keeping the two compared sets as nearly alike as possible often reduces individual variations in the results to a marked degree. As illustrations of such details may be mentioned (1) the alternating of individuals of the two series on the laboratory tables so that differences in external conditions due to position may be equalized, and (2) the handling of all specimens in the same manner at the time of the operation, including the unoperated as well as the operated ones. The first precaution in large measure removes differences due to temperature, to order of feeding, to

See Spallanzani 1769 and Davenport (1899).

Spallanzani speaking of the regeneration of the legs and tails says, "Yet when salamanders are kept fasting a longer time they begin to grow more lean and tapering than those that are fed. But the reproduction continues still in the same way."

<sup>10</sup> This journal, vol. viii, no. 3.

<sup>&</sup>quot; See this journal, vol. vi, no. 3.

degree of light, to movements of persons in the room, and to change in accuracy of the operation as a result of fatigue of the operator. The last is an important item when two hundred or more operations are made in a single day. As an example of the second precaution may be mentioned the mode of procedure in removing the tails of salamander and frog larvæ. The larvæ that were not to be operated upon were put out on the paraffine blocks with the others so that any differences due to the mere shock of transference might be equalized.

Direct sunlight was avoided in all cases, even a few minutes was sufficient in the case of salamander larvæ to seriously disturb the ordinary rate of regeneration.

Differences due to sex were not made out in the present experiments. In the few sets in which sex records were kept, no noticeable difference was made out between males and females.

Notwithstanding all the precautions that could be taken, individual variations in rate were present. The results were freed from this source of error in most cases by the use of a large number of individuals. In some sets the averages obtained are wholly reliable, as shown by the internal evidence of uniformity in the data: In other sets, especially those in which accidental death or injury has reduced the number of available individuals, the results are not so conclusive.

#### III EXPERIMENTS BY THE AUTHOR

The present paper includes the data on the regeneration of the following organs or parts:

- Group A. I The tail of the larval salamander, Amblystoma jeffersonianum Green.
  - 2. The margin of the disk in Cassiopea xamachana Bigelow.<sup>12</sup> Group B. 3 The chelæ of the crayfish, Cambarus bartoni.
  - 4 The chelæ of the gulf-weed crab, Portunus sayi.13
  - 5 The chelæ of the gulf-weed shrimp. Palæmon tenuicornis.
- 6 The chelæ of the Woods Holl shrimp, Palæmonetes vulgaris Stimp.

<sup>12</sup> Zeleny '07.

<sup>13</sup> Zeleny '08.

The non-molting and molting animals require fundamentally different methods of treatment and are separated in the following account.

# Experiments on Non-Molting Animals

The experiments upon the non-molting animals are simpler than those upon the others, because the rate of regeneration in animals with a definite molt varies according to the time within the period. The present experiments are therefore simpler than the others and less liable to danger from disturbing factors. The animals are, however, more subject to variation in the location of the plane of removal since the chelæ in the other groups have definite breaking joints.

## 1 The Tail of the Larval Salamander, Amblystoma jeffersonianum

Method. A large number of egg-masses in early cleavage stages were collected in a single pond on January 20, 1907 (except egg-mass J which was collected on January 8). These were brought to the laboratory and were put into separate dishes on January 25. The young when hatched were thus separated into groups, all the members of a group coming from one mass and presumably from the same female. The eggs began to hatch on January 31. On February 2 the hatched young were put into individual dishes and given egg-mass as well as individual numbers. Unhatched and deformed embryos were not used. One hundred and eighty-five larvæ belonging to egg-masses A, B, C, D, E, F, G, H, I and I were used in the experiment.

The general method consisted in the comparison during a definite period of the third or fourth regeneration of the tail of one set of salamander larvæ with the first regeneration of another set otherwise similar.

Sources of Error. I Age. The age factor was eliminated by making comparisons not between the successive regenerations of a single individual but between two sets of animals of the same age.

2 Periodic Physiological Changes. No special precaution apart from that used in connection with other factors is necessary in the case of salamander larvæ.

- 3 Differences in Laboratory History. All the egg-masses, with the exception of egg-mass J, had the same laboratory history. They were collected at one time from the same pond and had the same laboratory environment.
- 4 Changes in Rate During the Regeneration Period. Comparisons are made only during identical parts of the regeneration process.
- 5 Differences in the Level of the Cut. All cut surfaces from which successive regenerations were compared were made as nearly at the same level as possible. Differences between individuals could not, however, be avoided, (a) because of the activity of the larvæ, and (b) because the line between the old and new tissue sometimes becomes obscured. In connection with the former difficulty all animals with second or later cuts evidently away from the line of the first cuts were discarded. The latter difficulty is a more serious one, since a slight change in level involves not only a difference in itself, but also because it involves a difference between old and new tissue. Fortunately this source of error was possible in only a few individuals.
- 6 Additional Injury. All salamanders with additional injury were discarded. The precaution of putting the larvæ into separate dishes is necessary, not only because of the desirability of keeping separate records, but also because of the cannibalistic tendencies of the larvæ. When several larvæ are kept together most of the tails, legs, and gills are kept closely cropped.
- 7 Temperature. The larvæ were not kept at a constant temperature, but were all kept close together at one end of the room in order that the temperature of all dishes might be nearly equal. Furthermore, larvæ to be compared with each other occupied alternate positions on the table.
- 8 Food. All larvæ were fed the same number of Tubifex worms of a uniform size each day.
- 9 Individual Variation. This was kept down by the above major precautions in addition to the following minor ones. The dishes were of equal size and were all filled to the same level with water. To further insure the good health of the larvæ, dust was excluded by glass covers slightly raised at one edge and the water was changed every day.

Variations due to imperfections in the carrying out of the above precautions or to other neglected factors can largely be eliminated from the general result by the use of a large number of individuals. This was done fairly well though not as fully as desirable. While the original number was sufficiently large in all the series the rigid exclusion of larvæ that were influenced by accessory factors reduced this considerably in some. Most of these accidents were in the form of additional injuries which were especially likely to occur at the time of changing of the water. Though the number of valid individuals is not great in some of the single series the general result obtained from all the series is wholly reliable especially since all seven experiments are in entire agreement.

Data. The salamanders were divided into six series as follows:

TABLE 1—Amblystoma Jeffersonianum
Record of Series

SERIES	EGG MASSES	APPROXIMATE POR- TION OF TAIL REMOVED	REGENERATIONS COMPARED	LENGTH OF FINAL REGENERATION PERIOD IN DAYS
I a	A, B	2/5	third-first	14
I b	ΑВ	4/5	third-first	14
II a	C, D, E, F, G	2/5	fourth-first	11
II b	C, D, E, F, G	4/5	fourth-first	11
III a	H, I, J	2/5	fourth-first	10
III b		4/5	fourth-first	10
		<u>i </u>		

The dates of the operations in the different series are given in Table 2.

TABLE 2—Amblystoma jeffersonianum

Dates of Operations

SERIES	FIRST OPERATION	SECOND OPERATION	THIRD OPERATION	THIRD AND FIRST OPERA- TIONS		CLOSE OF EXPERI- MENT	FINAL RE- GENERATION PERIOD IN DAYS
I a— I b II a— II b. III a—III b.	February 17 February 22 February 23	March 13 March 9 March 10	March 24 March 25	April 6	April 9 April 10	April 20 April 20 April 20	14 11 10

In Series Ia and Ib the removed part was fully regenerated in the twenty-four days before the second operation was made. In Series IIa, IIb, IIIa, and IIIb between five and six-tenths of the length was regenerated in the corresponding period of fifteen days.

On the day when the third operation was made on a half of the members of Series Ia and Ib, the first operation at the same level was made on the other half. In Series IIa, IIb, Illa and IIIb, correspondingly the fourth and first operations were made together.

In Series Ia none of the individuals with a third regeneration survived the vicissitudes of the experiment. The data for the other five series are given in Tables 3, 4, 5, 6, 7 and 8. Lengths are in millimeters. The length of the removed part of the tail was obtained by subtracting the length of old tissue in the tail from the average length of the uninjured tail in a control series of salamanders. The individuals are arranged in order of length of the removed part of the tail. The specific amount equals the amount of regeneration per unit of removed tail length. The specific rate equals the amount of regeneration per unit of removed tail length per day.

TABLE 3a—Amblystoma Jeffersonianum. Series 1b. (Individual Data)
Approximately four-fifths of tail removed. Regeneration period equals fourteen days

NERATION	THIRD REGENERATION				
First regeneration	Length of removed tail	Third regeneration			
4.2	17.7	4.5			
17.9 3.2		5.3			
5.0	19.6	3.9			
5.1	19.6	5.0			
3.6		-			
4.22	19.00	4.67			
	4.2 3.2 5.0 5.1 3.6	First regeneration Length of removed tail  4.2 17.7 3.2 19.1 5.0 19.6 5.1 19.6 3.6			

TABLE 3b—Amblystoma jeffersonianum Series Ib. (Results)

	4	AVERAGE SPECIFIC	AVERAGE SPECIFIC
First regeneration—five cases		5	+ 0.0159 0.0176
Increase with successive removal Per cent of increase		0.023 10 per cent	0.0017

## TABLE 4a—Amblystoma jeffersonianum Series IIa. (Individual data)

Approximately two-fifths of tail removed. Regeneration period equals eleven days.

FIRST RF	GENERATION	FOURTH REGENERATION					
Length of removed tai	l First regeneration	Length of removed tail	Fourth regeneration				
7 3	1.0(-)	8.0	1.7				
7.8	1.0(-)	8.5	1.6				
8.9	2.6	8.8	2.5				
9.5	2.1	8.9	1.0(-)				
10.0	1.0(-)	8.9	2.0				
11.0	2.0	9.1	2.0				
11.2	1.0(-)	11.4	2.9				
8.11	2.5	12.1	3.8				
		13.6	1.3				
verage 9.69	1.65	9.92	2.09				

## 'TABLE 4b—Amblystoma jeffersonianum Series IIa. (Results)

	AVERAGE SPECIFIC	AVERAGE SPECIFIC
First regeneration—eight cases.  Fourth regeneration—nine cases.		0.0155
Increase with successive removal	,	+0.0037

TABLE 5a-Amblystoma jeffersonianum Series IIb. (Individual data)

Approximately fourth-fifths of tail removed. Regeneration period equals eleven days.

FIRST REC	GENERATION	FOURTH REGENERATION					
Length of Removed Tail	First Regeneration	Length of Removed Tail	Fourth Regeneration				
17.4	4.0	17.3	2.4				
17.8	2.0	17.6	3.1				
18.1	3.6	17.8	3.0				
18.3	3.3	17.8	3.7				
18.5	2.5	17.8	4.4				
18.5	2.8	18.1	3.8				
18.7	3.9	18.2	3.5				
19.9	2.0	18.5	3.9				
20.1	3.7	18.6	3.8				
ĺ		19.3	4.4				
1		19.5	3.5				
ì		20.0	3.8				
		20.4	4.8				
į		20.5	4.5				
		21.2	1.5				
,		21.2	4.4				
	•	21.3	5.0				
verage18.59	3.09	19.12	3.74				

TABLE 5b—Amblystoma jeffersonianum Series IIb. (Results)

<u>.</u>	AVERAGE SPECIFIC	AVERAGE SPECIFIC
First regeneration—nine cases		0.0151
Increase with successive removal	+ 0.030 18 per cent	+0.0027

## TABLE 6a—Amblystoma jeffersonianum Series IIIa. (Individual Data)

Approximately two-fifths of tail removed. Regeneration period equals ten days.

ENERATION	FOURTH REGENERATION					
First regeneration	Length of removed tail	Fourth regeneration				
1.6	6.2	1.0 (-)				
2.2	9.9	2.0				
2.2	10.1	2.2				
1.0(-)	10.4	1.9				
	10.7	2.7				
1.75	9.46	1.96				
	1.6 2.2 2.2 1.0 (-)	First regeneration Length of removed tail  1.6 6.2 2.2 9.9 2.2 10.1 1.0 (-) 10.4 10.7				

## TABLE 6b—Amblystoma jeffersonianum Series IIIa. (Results)

	AVERAGE SPECIFIC '	AVERAGE SPFCIFIC
First regeneration—four cases	0.190	0.0190
Fourth regeneration—five cases	0.207	0.0207
Increase with successive removal	+ 0.017	+ 0.0017
Per cent of increase	9 per cent	

## TABLE 7a—Amblystoma jepfersonianum Series IIIb. (Individual data)

Approximately four-fifths of tail removed. The regeneration period is ten days.

FI R 5 T	REGENERATION			FQ (	RTH	R	egene	RATIO
		 ٠,	 	 				

Length of removed tail	First regeneration	Length of removed tail	Fourth regeneration
16.7	3.0	0.81	3.2
17.7	1.5	18.1	4.1
18.0	2.8	18.5	3.8.
18.8	4.0	18.6	3.9
19.0	3.7	19.2	3.8
19.2	4.4	19.5	3.7
19.7	4-3	19.6	3.5
20.1	3.8	20.2	5.7
		20.3	4.4
		21.2	4.6
Average18.65	3.44	19.32	4.07

# TABLE 7b—Amblystoma jeffersonianum. Series IIIb. (Results)

. <del>.</del>		AVERAGE SPECIFIC	AVERAGE SPECIFIC
First regeneration—eight cases Fourth regeneration—ten cases		O 184	0.0184
Increase with successive removal Per cent of increase	.1	+ 0.027 15 per cent	+ 0.0027

General Result. Each of the five series shows a distinct advantage in rate of the fourth or third regeneration over the first. The average increase is fifteen per cent and is to be noted not only in the absolute amount regenerated, but also in the specific amount and the specific rate. The number of individuals in Series Ib and IIIa is too small to make these series conclusive by themselves, but in Series IIa, IIb and IIIb the number of individuals is sufficient to prove the fact of the increase. When all the sets are taken together there can be no doubt of the general result. The five series should not be averaged together for a strictly accurate determination because the factors involved are different in the different series. The special factors that differ are given in sources of error, Nos. 1, 4 and 6 on pp. 483 and 484. The average increase is put down in the tables merely as an indication of the general trend of the results.

# 2 The Margin of the Disk in the Scyphomedusan, Cassiopea xamachana<sup>14</sup>

For the method and other details the reader is referred to the original article. A brief statement of the results is given here for the sake of completeness.

The first regeneration of the outer margin in three individuals was compared with the second regeneration in two other individuals as shown in Table 9; the second regeneration is distinctly more rapid than the first. The considerable individual variation should, however, be noted.

<sup>14</sup> Journ. Exp. Zoöl., vol. v, no. 2, pp. 265-274, December, 1907.

TABLE 8-AMBLYSTOMA JEFFERSONIANUM. General Results.

	REGENERATIONS	NUMBER	NUMBER OF CASES	LENGEH	AVERAGE I REMOVED	AVERAGE LENGTH OF AVERAGE LENGTH OF REMOVED PORTIONS REGENERATED OF TAILS TAILS	AVERAGE LENGTH REGENERATED TAILS	E LENGTH OF ENERATED TAILS		SPECIFIC AMOUNT	SPECIF	SPECIFIC RATE	PER CENT OF
SIRIES	COMPARED	First Regenera- tion	First Third or Regenera- Regenera- tion tion		First Regenera tion	Third or Fourth Regenera-	First Regenera-	Thurd or Fourth a-Regenera-	First Regenera-	Thurd or Fourth Regenera-	First Fourth Regenera-Regenera- tion tion	Third or Fourth Regenera-	
I b	first—third	2	+	*	18.92	19 80	4.22	4.67	0 223	0.246		9210.0 6510 0	+ 10
II a	first-fourth	<b>∞</b>	6	=	6.69	26 6	1.65	7 00	0.170		0 0155	0 0155 0 0192	+24
H 6	first-fourth	<b>6</b>	17	=	18.59	19.12	3.09	3.74	991.0		8710.0 1510.0	8.0178	+ 18
III a	first-fourth	+	٠. س	2	9.22	9+6	1.75	1.96	0.190		0610 0	0.0207	6+
9 TIT	first—fourth	••	으 요	2	18.65	19.32	3.4	4.07	0.184	0.211	0 0184	0.0211	+15
Total		34	\$							i			
Difference		: : : : :	:	: :		: :			0.187		0.214 0.0168 0.0193	0.0193	+15

TABLE 9—Cassiopea xanachana
Width of regenerated margin in millimeters

FIRST REGENERATION	SECOND REGENERATION
0.5	0.8
0.2	1.6
0.2	i
Average = 0.3 mm.	Average = 1.2 mm.

In three individuals the first regeneration of a segment of the outer margin was compared with the second regeneration of a similar segment of the same animal. The method differed for the three cases and the original article should be referred to for the differences. In all three cases the second regeneration is more rapid than the first. The data are given in Table 10.

TABLE 10—CASSIOPEA XAMACHANA.

Width of regenerated margin in millimeters

INDIVIDUAL	FIRST REGENERATION	SECOND REGENERATION
A	. 1.6	1.8
3	. 1.1	2.0
C		1.4
	Average = 1.2 mm	Average = 1.7 mm.

The general result in Cassiopea xamachana shows distinctly an increase in rate of regeneration with successive removal. This is true not only when separate individuals are compared, but also when two segments within single individuals are used. The influence in this case seems therefore to be in part at least localized and not constitutional. The greater difference shown between the first and second regenerations in separate individuals as compared with the differences between segments of single individuals indicates that the effect of successive removal is also partly constitutional. The agreement of all the individuals as far as the general result is concerned should be emphasized. However, the small number of

individuals and the great individual variability cannot be neglected in an estimate of the validity of the conclusions, at least, as far as Table 9 is concerned. The data are thus open to the general criticism of insufficient control of the accessory factors.

# Experiments on Molting Animals

The second group of experiments includes those on Crustacea. A statement of the advantages and difficulties common to all of these may be given here. The chelæ were used in all four species for the study. The presence of a definite breaking joint enables the operator to be absolutely sure that he has the same plane of removal in all cases. This is a matter of great importance since it has been shown that the rate of regeneration is very closely related to the location of the plane of removal.<sup>15</sup>

An advantage due to the molting habit is the convenience in making measurements of the various stages in the process of growth and regeneration. The cast skin and the removed chelæ, as well as the preserved animals may be kept indefinitely in alcohol without danger of shrinkage and a complete record of the growth of all parts can be kept for future measurement. The cast must, however, be removed from the dish containing the animal soon after the molt, since it is eaten as soon as the mouth parts become sufficiently hardened to be capable of chewing.

The special difficulty with Crustacea is found in the same molting habit. It is not possible to use a definite period of time for the comparison because the amount of regeneration during such a period is dependent not only on its length, but also upon its relation to the molt. It is therefore most important that the operations be made at the same time with reference to the molt. Furthermore, in making comparisons of amounts of regeneration in individuals of approximately the same size or age it is necessary to use not a definite period of time, but a single molting period or a definite number of molting periods as a unit. This is true because it has been found that in individuals of the same size or age the

<sup>&</sup>quot; Ellis: '09, Morgan '06.

<sup>16</sup> Emmel '06; Zeleny '05.

molting period may differ considerably in length without greatly affecting the amount of regeneration taking place within the period. In other words, all or nearly all of the regeneration that is to occur during a molting period occurs during the first few days. It is obvious in connection with this statement that the rate of regeneration may be accelerated or retarded as far as the active regenerating period is concerned without any visible external evidences as long as the total amount regenerated during the molting period is unaltered. This possibility must be borne in mind in comparing data on the Crustacea with data on other forms.

# 3 The Chelæ of the Crayfish, Cambarus bartoni

Method. A female crayfish with young attached to her swimmerets was collected on December 14, 1906. The young, fifty in number, were kept together until February 11, 1907, when they were put into individual dishes. The water was changed frequently. The food consisted of Tubifex worms, the supply of which in each dish was never allowed to be exhausted. All shed skins were preserved in 85 per cent alcohol, and all operations were made two days after a molt. The autotomy of the chelæ was effected by injuring the nerve with a needle.

The experiment consisted in the repeated removal and regeneration of a single or both chelæ in about one-half the crayfish. The regenerating chelæ on the average attained about three-fourths of their original length before a new removal. No operation was made on the second half of the individuals until the first ones were ready to begin their third or fourth regeneration. Their chelæ were then removed for the first time. A direct comparison was thus made possible of the first and third or fourth regenerations. In the tables of data (Tables II and I2) the chela length is the length in millimeters of the propodite. The thoracic length is the length of the cephalothorax to the tip of the rostrum.

Because the molting periods did not coincide in the different crayfish only a small number of individuals is available for any one comparison.

The young crayfish were probably in their fourth molt when the observations were begun on February 11. The internal evidence of regularity of time of the first molt after February II indicates that all had molted the same number of times. Since there is no certainty as to the number of previous periods, the first molt after February II is called molt I, the second molt 2, etc. In the data of the experiments the period during which a regeneration occurred is indicated by its bounding molts. Thus period 6-7 indicates that the operation was made two days after the sixth molt and that the regeneration continued until two days after the seventh molt.

The specific amount of regeneration is the length of the regenerated chela divided by the final thoracic length or the length regenerated per millimeter of thoracic length. The specific rate is the length regenerated per millimeter of thoracic length per day. The specific rate as already stated (p. 493) is not a proper basis for comparison. The data are classified in the tables for direct comparison between the first<sup>17</sup> and third or fourth regenerations.

Sources of Error. 1 Age. All the individuals belonged to a single brood. However, since the crayfish did no molt at the same rate and since comparisons are made between corresponding molts and not between individuals of the same age, the age factor was not perfectly controlled. The error due to this source is, however, probably slight since no great differences in age exist.

- 2 Periodic Physiological Changes. Molting. This very important factor was controlled as far as possible by several devices.
  - a All operations were made two days after the molt.
  - b The molting period and not the day is used as a unit.
  - c Comparisons are made between corresponding periods.
- 3 Laboratory History. All individuals belonged to the same brood and had the same laboratory history.
- 4 Changes in Rate during the Regeneration Period. Because of the necessity of controlling the molting factor the time period is not the same in compared individuals. However, since all or nearly all of the regeneration comes in the early part of a molting period, and since whole periods were used as units in the comparison, this source of error is probably negligible.

- 5 Differences in the Level of the Cut. Because of the presence of a breaking joint in the chela no differences in level existed.
- 6 Additional Injury. Crayfish with additional injuries in any part of the body were discarded.
- 7 Temperature. Individuals belonging to different sets were alternated in their positions on the table and all the dishes were grouped together. In changing water the same amount of water was put in each dish.

TABLE 11—CAMBARUS BARTONI
One chela removed

First and third regenerations compared in periods 5-6 and 5 to 7. First and fourth regenerations compared in other periods.

		F1	RST REGE	NERATIO	N	THIRD OR FOURTH REGENERATION			
REGENERATIONS CONTARED	PERIOD	Final thoracic length	Length of regenerated chela propodite.	Regeneration period in days.	Specific amount of regeneration	Final thoracic length	Length of regenerated chela propodite	Regeneration period in days.	Specific amount of regeneration.
sst and 3rd {	5 to 6	11.3 11.7 12.4 12.9	5·3 5·7 4·4 2·7	26 30 20 21	0.47 0.49 0.36 0.21	11.0 11.0 11.3 11.6	4.2 5.3 4.6 5.4	24 31 23 33	0.38 0.48 0.41 0.47
. (	(	12.1	5.4	27	0.45	12.1	5·4 2·4	31	0.45
st and 4th	6 to 7	13.0	6.0	43	0.46	13.1	6.0	26	0.46
7 to 8					13.9	6.7 6.7	28 30	0.53	
et and 3rd {	5 to 7	12.4	6.2	39	0.50	11.0 12.0 12.3	4.9 6.3 6.2	35 68 48	0.45 0.525 0.50
						12.6 12.9 13.0	6.4 6.7 6.7	54 65 71	0.51 0.52 0.52

- 8 Food. A superabundance of food was always at hand in each individual dish.
- 9 Individual Variation. An attempt was made as in the salamander series to keep the minor details alike in all cases. There is, however, a considerable amount of individual variation. Unfortunately the peculiar necessity of grouping individuals which results from the molting habit makes the number of individuals in any single comparison much smaller than is desirable.

Data. In Table 11 all but two of the seven first regeneration individuals have a specific amount of regeneration between 0.45 and 0.50. Likewise, all but three of the fifteen third or fourth regeneration individuals have specific amounts between 0.45 and 0.53. The exceptional individuals are all below the ordinary level and may be explained on the basis of infection of the wound or other uncontrolled factor in the conditions of the experiment. Similarly in Table 12 no striking difference between the rates is apparent.

TABLE 12—CAMBARUS BARTONI

Both chelæ removed

		FIRS	TION SECO		THIRD OR FOURTH REGENER				
REGENERATIONS COMPARED.	PERIOD	Final thoracic length	Length of regenerated chela propodite.	Regeneration period in days.	Specific amount of regeneration	Final thoracic length	Length of regenerated chela propodite	Regeneration period in days.	Specific amount of regeneration.
ıst and 3d	5 to 6 {	11.3 11.6 11.9	5.05 4.3 5.45 5.95	22 23 27 . 25	0.45 0.37 9.46 0.46	11.5	5·4 5·3	30 26	0.47 0.45
2d and 4th	6 to 7	12.6	5.1	19	0.40	13.0	5.5	22	0.43
1st and 4th	7 to 8	13.6	6.7	30	0.49	-	_		
1st and 3d	5 to 7					12.4	6.75	52	0.54
	-					12.8	6.7	47	0.52

General Result. The general result thus shows that successive removal brings about no change in the rate of regeneration. If the data indicate any change it is in the direction of increase and not of decrease. While the separate periods should strictly not be subjected to a cross comparison because of differences in the factors involved it is interesting to note that a general average of the specific amounts in each table gives the advantage to the later regeneration, not only when all the individuals are included, but also when the exceptional individuals in each column are eliminated. When the few exceptional individuals are removed from consideration the uniformity in specific amounts in the different cases is striking and the validity of the result is assured.

In conclusion then the statement may be made that in young crayfish of the species Cambarus bartoni of thoracic lengths between 10.1 mm. and 13.9 mm., successive removal of a chela has little or no effect upon its rate of regeneration for the first three or four removals. If any change is indicated by the data, it is in the direction of increase and not of decrease.

## - 4 The Chelæ of the Gulf-weed Crab, Portunus sayi

The method, data and results have been published in Tortugas vol. ii of the publications of the Carnegie Institution of Washington.<sup>18</sup> A summary of the results is given here for comparison with the other groups.

The method of procedure differs from that of the crayfish experiments. Individuals varying in size from 3 mm. to 15 mm. were collected and successive removals of the right chela were made in all the individuals. These removals came in each case on the day after a molt. Apart from the age factor which was treated as described below the sources of error were treated in essentially the same manner as in the case of the crayfish (p. 495). For further details the original account should be consulted.

It was found that in sixteen out of twenty cases the second regeneration was greater than the first. In three the reverse was true

and in one the two were equal. In the two cases with a third regeneration the third was greater than the second. This statement, however, does not consider a possible change with age. When the age factor is eliminated the difference disappears. In individuals of equal cephalo-thoracic length the amount of the first regeneration is approximately equal to that of the second. The average specific amount of the first regeneration is 0.791 for sixty-six cases and of the second regeneration 0.789 for twenty-five cases. The difference between the two is well within the limit of probable error. Therefore it may be concluded that successive removal of the right chela in the gulf-weed crab ne ther retards nor accelerates its power of regeneration.

# 5 The Chelæ of the Gulf-weed Shrimp, Palæmon tenuicornis

Method. Twenty-four gulf-weed shrimps were collected on June 16, 1906, at Tortugas. They were put into individual dishes and fed every day on pieces of a small fish, Atherina. The operations consisted of the removal of a single chela (the right in all except No. 1442) on the day after a molt. The general mode of procedure agrees closely with that for the gulf-weed crab. However, on account of the small number of individuals used, it was not possible to entirely eliminate the age factor. Accordingly the comparisons as given here are between the successive regenerations of a chela within single individuals and are not valid in a general discussion of the effect of successive removal as a distinct factor.

Data. Regeneration was approximately completed within a single molting period. Cases which ran for two molting periods or which had an additional injury are indicated by an asterisk (\*) in Table 13. The measurements of the chelæ are the lengths in millimeters of the propodites. The thoracic length is the post-spinous thoracic length. The specific amount of the last regeneration is the regenerated chela length divided by the final thoracic length or the amount of regeneration per unit of thoracic length.

Results. An examination of Table 13 shows that in individual shrimps with two exceptions there is a gradual decrease in the

<sup>19</sup> Zeleny '08,

TABLE 13—PALAEMON TENUICORNIS

Successive regeneration of the chelæ

CATA- LOGUE NUMBER	ORIGINAL LENGTH CHELA PROPODITE	FIRST REGEN- ERATION	SECOND REGENERATION	THIRD REGEN- ERATION	FINAL THOR- ACIC LENGTH	SPECIFIC AMOUNT OF LAST REGEN- ERATION
1429		2.22*	1.81			
1432	2.15	2.40	2.31	2.01	6.8	0.296
1434	2.41	r.88	2.02	1.89		
1436	2.38	2.01	1.96*			
1439	2.85	1.33	2.81		8.0	0.351
1440	2.60	2.44				
1441		2.78*	2.62	2.52	7.4	0.341
1442	2.96	2.74	2.56	2.39	7.6	0.314
1443	2.96***	2.73				
1444	3.30	2.96				
¥445		3.78*	3 · 44**	3.63	9.7	0.374
1446		3 · 57*	3 · 55	3.46		
1447	4.57	4.51	3.80*			
11448	4.07	3.70				
1449		4.11*	3.63	3.46	10.6	0.326
1450	3.73	3.14	3.19	3.12	11.0	0.284
1451	5.07	4.40	4.20		11.8	0.356
E452	_	5-55*	5.08	5.00	13.1	0.382

amounts of regeneration following successive removals. Because of the small number of individuals in the final comparison and because of the large amount of variation, it is not possible to determine the relation between the size of the individual and its amount of regeneration. The specific amounts of regeneration show no close relation between size or age and specific amount of regeneration. The molting data (Table 14) show on the average a decrease in length of the molting period during the time covered by the experiment, though the exact amount of its influence in the present case is hard to estimate. In Table 14 the two periods followed by a dagger (†) are ones in which the operation was made on the day after capture of the animals, the time of the previous molt being unknown. They should not strictly be included in the comparison because it is known that the effect of an operation upon the length of a molting period varies with the time of the operation in relation

TABLE 14-PALAEMON TENUICORNIS

### Molting Periods

	PERIOD	DURING WI	HICH THE REGENERA IN DAYS.	ATION OCCURRED,
CATALOGUE NUMBER		Regenera- tion	Second Regenera- tion	Third Regenera
1432		9	9	8
1441	.	15†	9	10
1442		10	10	9
1445		16†	11	8
1446		10	10	10
<b>1447</b>	.	13	11	
1449	.  :	13	11	11
1450	, į	8	9	8
1451	·i	9	12	
1452		13	10	10
Average		10.6	10.2	9.25
	8 0	ases	IO Cases	8 cases

to a molt. The irregularity in these shrimp data is probably due in large part to the habits of the animals. The water in the dishes had to be changed every day and it was impossible to do this without greatly disturbing the shrimps which in their violent struggles were often considerably bruised. Such injuries are difficult to estimate.

The data under the present head thus leave one in doubt as to the effect of successive removal as an independent factor because of the small number of individuals and the lack of control of the age and other factors. In speaking merely of the amount of regeneration after successive removals of a single chela without reference to the analysis of the factors involved, the statement may be made that in shrimps of the species Palæmon tenuicornis with thoracic lengths between 6.8 mm. and 13.1 mm. the amount of regeneration decreases with successive removals.

6. The Chelæ of the Wood's Hole Shrimp, Palæmonetes vulgaris, Stimp.

Method. In the case of the Wood's Hole shrimp the object of the experiment was the comparison of the rate of the first regeneration of the right chela with the simultaneous second regeneration of the left chela and vice versa. The advantage of this method consists in the elimination of differences between individuals. The principal source of error is the possibility of inherent physiological differences between the two chelæ. This error is in all probability negligible because there is little evidence of a structural difference between the two chelæ though the right is on the average slightly larger than the left. Since the left chela was removed in twenty-five individuals, and the right in twenty, constant latera. differences are practically balanced. The only error is that of possible individual lateral differences and this is eliminated by the large number of shrimps used.

A peculiarity of this method as compared with the others, except Cassiopea, should be emphasized. The result cannot be directly compared with the results of the previous series on the crayfish. If the effect of the removal of a chela as far as rate of future regeneration is concerned is a purely local matter in olving merely the immediate neighborhood of the wound the comparison is valid. If, however, as we have many reasons for believing, the effect of the operation involves the general physiological condition of the animal as a whole the first removal and regeneration of a chela may have as much influence upon the future regeneration of the opposite one as upon its own second regeneration. If this be true we may expect the two rates to be equal whether the general effect of successive removal be positive, negative or indifferent.

Fifty-one shrimps of approximately equal size were selected from the smaller individuals of a lot of about three hundred collected on August 15, 1906, at Wood's Hole, Mass.<sup>20</sup> Forty-five of these were used in the present experiment and thirty-one are valid

<sup>&</sup>lt;sup>30</sup> I am indebted to Mr. Sergius Morgulis for kindly consenting to take care of the shrimps after my departure from Wood's Hole. Without his help I should not have been able to complete the experiment.

for present comparisons, the remainder being discarded because of death, escape or accidental injury. The animals were kept in individual numb red finger-bowls. Food consisting of pieces of Fundulus was supplied every day except August 17, and the water was changed every day. The first operations were made on August 15th. The order of procedure was as follows:

- a The right chela was removed in twenty individuals, and the left in twenty-five individuals.
- b Both right and left chelæ were removed on the day after the first molt which gave a nearly complete regeneration of the removed organ. In some cases this was the first, in others the second molt. About seven-tenths of the original length was ordinarily regenerated at the time of a second removal.
- c On the day after the next succeeding molt, the animals were preserved in 95 per cent alcohol, and the right and left chelæ were compared.

The measurements used in the present comparison consist of the final thoracic length to the tip of the rostrum, and the final chelæ lengths, the second regeneration on one side of the body and the first on the other. The lengths given for the chelæ are the lengths of the propodites in millimeters.

Sources of Error. Because of the method of procedure most of the sources of error enumerated under the other experiments were eliminated, but at the expense of a restriction in the application of the results as pointed out above (p. 502).

- I. Inherent psychological difference between the two chelæ of an individual. This is eliminated by the use of a large number of individuals with the second regeneration nearly equally distributed between right and left chelæ. There are seventeen first and fourteen second regenera ions of the right chela and fourteen first and seventeen second regenerations of the left chela.
- 2 The molting habit. The successive removal may affect the rates at certain parts of the regeneration process without exerting an influence upon the total amount regenerated during a molting period. For instance, if after a first removal the whole regeneration to be accomplished during the molting period is practically completed during its first half, the effect of successive removal may

be to retard or accelerate this rate without any influence upon the total amount regenerated. Thus it may be accompolished in two fifths or three-fifths of the period of the molt which would

TABLE 15-PALAEMONETES VULGARIS STIMP.

Comparison of the second regeneration of one chela with the first regeneration of the opposite chela. Individuals arranged in order of thoracic length.

	FIRST REG	ENERATION	SECOND RE	BENERATION
FINAL THORACIC LENGTH MMS.	Side of body	Length of chela propodite	Length of chela propodite	Side of body
7.2	right	1.93	1.93	left
8.9	left	1.45	1.67	right
9.5	left	1.32	1.31	right
9.8	right	1.93	1.89	left
9.9	right	1.96	1.87	left
9.9	left	1.63	1.67	right
9.9	right	2.40	2.39	left
10.0	left	2.07	2.02	right
10.0	right .	1.78	1.78	left
10.0	right	2.71	2.71	left
10.1	right	2,20	2.19	left
10.1	left	2.51	2.43	right
10.2	left	2.29	2.27	right
10.2	right	. 2.21	2.20	left
10.4	left	2.35	2.30	right
10.7	left	2.25	2.27	right
10.7	right	1.70	1.62	left
10.9	left	1.73	1.65	right
61.0	right	1,96	2.07	left
11.0	right	2.60	2.48	left
11.0	right	2.64	2.52	left
11.0	right	2.71	2.63	left
11.1	left	2.27	2.17	right
11.1	. right	2.22	2.12	left
11.2	right	2.59	2.35	left
11.5	left	2.20	2.22	right
11.5	left	2.50	2.41	right
12.0	right	2.67	2.65	left
13.0	left	2.25	2.27	right
13.2	left	2.27	2.26	right
13.6	right	2.97	2.88	left
verage		2.20	2.17	
ifference		+ .03		
er cent of difference	· · · · · · · · · · · · · · · · · · ·	+1.4		

mean an acceleration of the rate in the first case and a slowing of the rate in the second case as compared with a first removal.

Data. The data are given in Table 15 which includes in the first column the final thoracic lengths, in the second and third columns the first regenerations and in the fourth and fifth columns the second regenerations.

General Result. In the thirty-one valid individuals, the average length for first regenerations is 2.20 mm. and for the second 2.17 mm. an advantage in favor of the first regenerations of 0.03 mm. or 1.4 per cent. This difference, though very slight, may be valid when we consider that in twenty-two of the thirty-one cases, the first regeneration is greater than the second. In only six is the reverse true while three are equal.

### IV DATA FROM OTHER SOURCES

The data from the literature of the subject may be grouped under two heads:

- I Data bearing on the effect of successive removal on the rate of regeneration, which is of special interest in connection with the present paper.
  - 2 Data showing an effect upon other features of regeneration. The first may be subdivided into three heads:
- a Data showing no change in rate as a result of successive removal.
  - b Data showing a decrease in rate with successive removal.
  - c Data showing an increase with successive removal.
- 1-a The first of these are by far the most frequently mentioned, though without the data giving actual measurements. The general statement is made that an organ may be removed and regenerated several times in succession without influencing its rate of regeneration. Spallanzani (1769) says: "If the four reproduced legs be cut off again, four new legs will make their appearance the second time as they did the first; and this is repeated several times. In fact, when the salamanders were quite young, at which time the reproduction is soonest performed, I have obtained in the course of the months of June, July and August, six successive reproduc-

tions of the four legs, and an equal number of successive reproductions of the tail at the same time. In one of these salamanders the reproduced bones of the legs and tail amounted in these three months to 687. This number of reproductions seemed not to have lessened in any considerable degree the regenerating power, as the last was obtained in the same number of days as the former. As this power manifests itself from April to September, it is very probable that, by beginning these sections in April and continuing them during all that time, one might obtain in these six months twelve reproductions both of the legs and tail."

- 1-b The second group of statements showing a decrease cannot be relied upon in the absence of proof that the whole physiological activity was not declining as a result of causes quite apart from removal.
- Vanlair ('94) finds in the sciatic nerve of the dog that the second regeneration is more rapid than the first, occurring in seven and a half months as opposed to ten months for the first regeneration. He says "Une autre remarque faite sur un de mes chiens mérite également de fixer l'attention. Ayant pratiqué sur un sciatique déjà régénéré ne seconde section à 1 centimètre au-dessus du premier niveau, j'ai pu constater que le deuxieme restauration 'accomplissait en un temps sensiblement plus court que la première. Alors que celle-ci avait exigé un délai de dix mois, l'autre n'a demandé que sept mois et demi. Il semble, d'après cela, qu'un prem er effort de régénération rende plus facile le travail nécessaire à une restauration ultérieure."

The subcutaneous tumor known as a keloid growth is said to regenerate more rapidly the more often it is removed.

- 2 The data showing an effect upon the character of the regeneration apart from rate may be grouped in the same way: (a) cases in which no change is noticed, (b) cases in which a change is shown.
- 2-a The general statement is made that successive removal has no effect upon the character of regeneration. (Spallanzani (1769) and others.)
- 2-b On the other hand there are some cases on record showing a change in character with successive removal. Thus Driesch

('97) finds in Tubularia that the character of the regenerated part, polyp or root-stock, is changed from one to the other with successive removal.

### Conclusions Regarding Data from the Literature

In general the facts are not reliable (first) because of the vague and general manner in which the statements are made implying merely an astonishment because the successive removal showed regeneration at all, and (second) because the additional factors likely to control the result such as age, temperature, etc., are not considered. The general concensus of opinion seems, though with a great deal of hesitation, to favor the view that successive removals followed by regeneration do not change the rate or other characteristics of the regeneration. The most striking exception is that of Vanlair ('94), who finds a distinct advantage in favor of the second regeneration of the sciatic nerve of the dog as compared with the first.

# V GENERAL STATEMENT OF THE FACTS AS OBTAINED FROM ALL SOURCES

The determination of the effect of successive removal upon the rate of regeneration is not a simple matter. Before proceeding further, it is necessary to emphasize a few points that have an important bearing on the interpretation of the data.

- I Special emphasis should be placed on the fact that every disturbing condition affecting the physiological state of an animal is a factor causing decrease in rate with successive removal. Hence, a result showing an increase or no change in rate must have an additional weight from this fact alone. At least nothing is more certain than that poor physiological condition, even if present in small degree and affecting both series of animals alike, must yield a result giving decrease in rate.
- 2 A factor whose effect is in part at least constitutional cannot be expected to have either uniformity or considerable magnitude.
- 3 In the determination of a factor whose value is less than that of a considerable number of other factors all of which are operating

at the same time, it is obvious that only a very careful control of these factors can give results showing any uniformity. In dealing with a factor like level of the cut, the difference in rate with level is greater than differences due to variation in the other factors and a more uniform result can obviously be obtained.

4 The literature of the subject does not take into consideration these subsidiary factors and accordingly cannot be used in a general statement. The age factor has been shown to indicate an increase in rate of regeneration with age in certain species in opposition to the general rule of decrease with age. The results so far obtained therefore show that it is at present unsafe without further evidence to say that the age factor is a minus or plus or negligible quantity. The statement in the literature that the rate of regeneration is uninfluenced by successive removal is unreliable as far as pure effect of removal is concerned. It is, however, probable that Vanlair's data would remain unchanged even with the elimination of the age factor, though his data are too meager for this purpose.

Taking the present data into consideration and making a comparison with former data, the following general statement of the facts seems warranted.

Among species without a molting habit the subsidiary factors are more readily eliminated than among those with a molting habit. The former show an increase in rate with successive removals (Vanlair, sciatic nerve-dog; Zeleny, tail-salamander and margin of disk—Cassiopea). Furthermore, the meager data from Cassiopea indicate that this effect is both constitutional and local.

Among species with a molting habit the complex molting factors are hard to eliminate and may yield some factors as yet wholly unknown. Eliminating sources of disturbance as far as possible it is found that successive removal of the chelæ causes little or no change in the rate of replacement.

The data as a whole therefore make it highly probable that the pure effect of successive removal is either no change in rate of regeneration or an increase in rate.

#### VI DISCUSSION

The data at hand show that successive removal during the first four removals does not diminish the rate of regeneration. It is either the same throughout or is visibly increased. For non-molting cases which seem to be the better adapted for an accurate determination the rate is increased with successive removal. In molting cases it is apparently not changed.

Leaving out of consideration any general discussion of the facts as an instance of adaptive response on the part of the organism two points deserve attention. The first concerns the mechanism of the process and the second involves the general bearings of the results.

- I The removal of an organ according to the generally accepted view is followed by the proliferation of partly or wholly undifferentiated cells near the cut surface. This proliferation is accompanied by degenerative changes in the differentiated cells and makes up the early slow period of regeneration. It is followed by a period of very rapid multiplication and resulting rapid growth. Then, finally, with the differentiation of the cells the rate gradually diminishes. If the new organ is removed for a second time before the completion of the old process the second organ grows more rapidly because the proliferating cells are already present, and the conditions for new growth are more favorable. This, however, does not explain an advantage, if any, that the third or later regenerations may have over the second.
- 2 The special interest of successive regenerations is largely in connection with the problem of the limitation and cessation of growth. In the development of most organisms, growth follows a definite cycle with a maximum rate near its beginning followed by a rapid decrease and finally a more gradual decrease ending in cessation. We are not in a position at present to discuss the probable causes of this limitation, but it is known that certain kinds of injuries such as those involving the removal of a part of the body bring about a renewal of the process. The new organ in its growth follows the same cycle as before. It is not possible, however, in this case to make a comparison of the absolute rate with that of

normal growth because the conditions are so entirely different. The only thing that can be made out is that the replacement follows in general the same rate changes as in ordinary growth. In the case of successive replacements, however, it is possible to make a direct comparison. Here a test can be made of the general question of growth potential. Does the accomplishment or partial accomplishment of the normal growth process or of its first repetition imply the using up of an original supply of growth potential? If it does, should it not be natural to infer that a history of successive removals and replacements ought to be followed by less rapid and less perfect regeneration? It does not matter whether this growth potential is put in terms of chromatin units, accessory germs or any other kinds of physical units or units of "entelechy" efficiency. The fact remains that regeneration occurs as readily or more readily after previous replacements than before. The organism does not act as if it had used up any of its essential supplies. Rather it shows a tendency to a more rapid performance upon previous trial.

The facts seem thus to point strongly toward external rather than internal initiative in the causation of growth limitation. There is an inexhaustible supply of material within the organism for the production and reproduction of its parts. The undiminished force of the developmental processes during successive regenerations shows in a very obvious way that the cause of restriction and cessation of growth is to be sought in the presence of certain relations between the parts of the organs or between them and outside forces and not in the absence of any specific materials necessary for further growth. When the proper relations are produced by injury, growth starts again with undiminished force.

### VII SUMMARY

- I The rate of successive regenerations was studied with special reference to the determination of the pure effect of successive removal after elimination of the accessory factors.
- 2 In the best controlled series, it was found that successive removal does not cause a decrease in rate of replacement, but either no change or an increase in rate.

- 3 In one group of experiments the effect of successive removal as a whole was studied. In the other group the local effect only was determined.
- 4 In the larval salamander the experiment was well controlled and showed in each set an advantage in favor of the later regenerations.
- 5 In Cassiopea the number of individuals was insufficient to make the result absolutely certain, but each of the later regenerations was distinctly in advance of the first.
- 6 In crayfish and gulf-weed crabs there was no change with successive removals. Both animals furnish a special difficulty in the shape of the molting habit, but this was controlled as perfectly as possible.
- 7 In the case of the gulf-weed shrimp Palæmon tenuicornis an important factor, the age factor, was not eliminated, and while in single individuals there was a decrease in rate of regeneration with successive injury, this may have been due to normal age decrease. The number of individuals also was insufficient.
- 8 The local effect was studied in the chelæ of the Wood's Holl shrimp, Palæmonetes vulgaris Stimp, and in the margin of the disk in Cassiopea xamachana.
- 9 The chelæ of Palæmonetes showed a slight advantage of the first regeneration over the second, but since it amounted to only 1.4 per cent of the length regenerated it may not be significant.
- 10 The margin of the disk in Cassiopea xamachana showed an advantage of the second regeneration over the first, but not as pronounced a one as in the cases in which the whole constitutional effect was in question.

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# THE RELATION BETWEEN DEGREE OF INJURY AND RATE OF REGENERATION—ADDITIONAL OBSERVATIONS AND GENERAL DISCUSSION<sup>1</sup>

# CHARLES ZELENY

In some recent papers<sup>2</sup> I have given the results of experiments which show an increase in rate of regeneration of an organ with increase in degree of injury to the individual. This relation does not keep up indefinitely but sooner or later an optimum degree of injury is reached beyond which additional injury results in a decrease in rate. In some cases the optimum is comparatively high, in others, comparatively low.

Realizing the many factors that control rate of regeneration and the difficulties encountered in attempting to eliminate all but one of them I have extended the experiments with two points in view, (1) the more careful control of the accessory factors, and (2) the inclusion of as many different forms as possible. The new results together with a summary of the old, make up the subject matter of the present paper.

### GENERAL METHODS AND SOURCES OF ERROR

Since in any particular case the rate of regeneration is determined by a large number of factors, many of which give marked changes in rate with slight changes in the factors, success in obtaining the pure effect of degree of injury is dependent upon the completeness of elimination of the other factors. The principal method employed was chosen with this point in veiw. It consists of a study of the change in rate of regeneration from a particular level when other parts of the individual are subjected to different degrees of injury. In the present paper by effect of

<sup>&</sup>lt;sup>1</sup> Contributions from the Zoölogical Laboratory of Indiana University. No. 109.

<sup>&</sup>lt;sup>2</sup> See Bibliography.

degree of injury is meant the effect of additional injuries to the individual. Special emphasis needs to be laid on this fact to avoid confusion in interpretation. A comparison of the rate of regeneration from different levels of a particular organ is not of direct value in connection with this problem because difference in level involves a great many factors not connected with degree of injury to the individual as a whole. The local conditions at different levels are obviously different and these differences have no necessary connection with degree of injury. Furthermore level cannot be assumed to be a measure of degree of injury, especially in the case of long, slender organs.

The lack of knowledge of the several subsidiary factors influencing the rate of regeneration makes it impossible to compare the changes in rate within single individuals. In every case it is necessary to compare the rate in different groups of individuals, each group being as nearly like the others as possible except in the degree of injury. The success of the method is dependent upon the similarity between the different groups in all respects except the factor under consideration.

The principal sources of error arise in connection with the following factors:

- 1 Age.
- 2 Periodic physiological changes.
- 3 Character of the laboratory history.
- 4 Changes in rate during the regeneration period.
- 5 Level of the cut.
- 6 Successive regenerations.
- 7 Temperature.
- 8 Food.
- 9 Differences in manipulation.
- 10 Departure of the living conditions from the optimum.
- The relation of the degree of injury to the optimum degree.
- 12 Individual variation.

The general methods employed in the elimination of these sources of error are based upon the attempt to make every two

compared groups with different degrees of injury as nearly alike in all other respects as possible. Each of the disturbing factors is taken up in turn.

- I Age. It is necessary to have the individuals of two compared groups of the same age, of the same brood if possible, because it is known that rate of regeneration changes with age. According to general belief rate decreases with age but exceptions to this rule are not unknown.<sup>3</sup>
- 2 Periodic physiological changes. In the Arthropods in general the outer covering is cast off at intervals and it has been shown that growth and regeneration have a very intimate relation to this habit. In Crustacea especially it has been determined that the time of the operation with respect to the molting period is of importance.<sup>4</sup> In experiments on Crustacea it is therefore necessary to take special pains in making the molting habit the center for adjustment of the compared groups. Notwithstanding the greatest care taken it is not possible to wholly eliminate the molt as a disturbing factor. The data dealing with animals having a molting habit are separated from those dealing with animals not having a molting habit.
- 3 Character of laboratory history. It is known that animals transferred from their natural environment to the laboratory show changes in their behavior. These changes are usually associated with a general decrease in vitality. It is therefore necessary to have the compared groups with identical laboratory histories.
- 4 Changes in rate during the regeneration period. Following an operation the rate of regeneration is not the same for the whole time up to the completion of the organ. In the frog tadpole and probably in other forms it is at first slow, then increases rapidly to a maximum, then decreases, rapidly at first, and then more and more slowly to zero at the time of completion of the regeneration.<sup>5</sup> It is necessary in our present experiments to compare the rates in identical periods only.
  - 5 The level of the cut. The rate of regeneration varies with

<sup>\*</sup> See this journal, vol. vii, no. 3.

<sup>4</sup> Emmel, 1904.

<sup>&</sup>lt;sup>5</sup> See this journal, vol. vii, no. 3.

the level of the cut. In the frog tadpole's tail Ellis has shown that the amount of regeneration in length except for the first few days following an operation is directly proportional to the length removed. Special care must therefore be taken to have the levels from which regeneration is to be compared as nearly identical as possible.

- 6 Successive regenerations. In a number of cases<sup>7</sup> it has been shown that the rate of regeneration changes with successive removal. It is therefore necessary to make comparisons for the effect of degree of injury between such groups only as have first regenerations of the organ in question. Second and succeeding regenerations may also be compared but the danger from disturbing factors is greater than in the first.
- 7 Temperature has a very direct effect upon growth and regeneration and it must be carefully controlled. It is probable that in some cases at least the influence of temperature upon rate of regeneration is more marked even than upon rate of ordinary growth.
- 8 Food. Morgan has shown for salamanders that abundance of food has very little if any influence upon rate of regeneration in length of the tails. In a study of the frog tadpole Miss Durbin has however found that change in quantity or in character of food influences the rate. In the present experiments compared sets were fed alike. A source of error that could not be controlled is due to the fact that individuals with the greater injury are frequently handicapped in catching or manipulating their food. When present this error probably favors the animals with the lesser degree of injury.
- 9 Differences in manipulation. The extra handling to which individuals with the greater degree of injury are subjected may in itself be harmful apart from the removal of the appendages. This is especially true of aquatic animals that must be operated upon in the air. It was frequently seen that individuals with the

<sup>6</sup> See this journal, vol. vii, no. 3.

<sup>7</sup> See this journal, vol. vii, no. 3.

Morgan, 1906. The physiology of regeneration.

See this journal, vol. vii, no. 3.

greater injury did not recover their ordinary activities as soon as those with the lesser injury.

- Departure of the living conditions from the optimum. If the two compared groups are exactly alike in original choice of animals and in treatment there is still a source of error that must not be neglected. The object in view is the determination of the effect under optimum living conditions. If the living conditions are poor it is evident that one group may suffer more than Under poor conditions such as foul water, presence the other. of pathogenic organisms, insufficient food, etc., it is natural to suppose that an individual with a greater degree of injury is likely to suffer much more than one with a lesser injury. The same may be said of differences inherent in the experiment, such as loss of blood, nervous shock, and the greater difficulty experienced by individuals with the greater injury in seizing and handling their food. Therefore under identical treatment of the two compared groups the series with greater injury is at a disadvantage whenever there is any departure from optimum living conditions.
- It Relation of the degree of injury to the optimum degree. It is obviously true that with a degree of injury so great that the animal is barely able to survive the operation a rapid rate of regeneration cannot be expected. Accordingly near the upper limits of degree of injury a decrease in rate is found. The general question therefore resolves itself into the location of the optimum degree of injury. Is it coincident with a very low, a very high or with a medium degree of injury? If we compare two groups of animals with different degrees of injury both of which are near the upper limit of degree the result will be different from that obtained near the lower limit of injury. Furthermore if the lower injury is below and the upper injury above the optimum there may be no difference in rate of regeneration. In most cases the present experiments deal with medium degrees of injury.
- 12 Individual variation. In most experiments differences classed as individual variations are due not to any inherent differences in the individuals themselves but to uncontrolled differences in treatment or in age of the animals. The principal sources of

error have been removed in the present experiments in the ways indicated above. The individual differences that remain are partly at least due to inherent differences. This source of error can be eliminated only by the use of a large number of individuals in each group. In the following data after throwing out all cases coming into conflict with the disturbing factors given above the number remaining for comparison is sufficient in some cases while in others it is unfortunately insufficient for conclusive results.

The data for each experiment are treated separately. New observations are given in full and the results of former observations in brief. The data are presented in two divisions, the first including animals without and the second those with a molting habit.

### EXPERIMENTS ON ANIMALS WITHOUT A MOLTING HABIT

Animals without a molting habit, as compared with those possessing this habit have a distinct advantage due to the absence of this complicated factor. They however have in most cases the disadvantage of less constancy in the level of the injury from which regeneration takes place. The level of the cut greatly influences the rate of regeneration and special attention was therefore paid in all non-molting cases to its elimination as a source of error.

### I The Opercula of Hydroides dianthus 10

The Serpulid worm, Hydroides dianthus, has a large, so-called functional, operculum on one side of the head and a small rudimentary operculum on the other side. Regeneration of the small operculum when it alone is removed is compared with its regeneration when the other or functional operculum is removed at the same time. In the first instance a new, small operculum gradually grows out from the cut surface. In the second instance the small operculum rapidly regenerates into a large one in case the func-

<sup>10</sup> Roux's Archiv, 1902 and Journal Exp. Zoöl., 1905, pp. 18-77.

tional operculum has been removed at the proper level. Thus with an additional injury to the individual the regenerating bud produces an organ larger in size and different in character from the original one. In my former papers I was concerned merely with the final results of the operations. A reference to my original notes however shows that there is a striking advantage in favor of the greater injury not only in the final size but also in the rate at which the material of the new operculum is proliferated. The same result was found in other species of Serpulids with dissimilar opercula such as Hydroides pectinata, H. uncinata, Serpula vermicularis, Apomatus ampullifera, etc.

# 2 Rapidity of Differentiation of the Opercula in Apomatus ampullifera<sup>11</sup>

The branchiæ of the Serpulid Apomatus ampullifera are arranged in two semi-circular groups one on each side of the mouth. One of the branchiæ has a large terminal bulb which serves as the functional operculum. The corresponding branchia of the opposite side has a slight enlargement. Both circlets of branchiæ are readily thrown off at a breaking joint near their bases. In fact it is scarcely possible to operate on the animals without producing this autotomy. When the animal is otherwise uninjured the two circlets of branchiæ are regenerated as described below. The two opercular branchiæ begin to show the terminal enlargements comparatively late in regeneration being at first wholly similar to the other branchiæ. When however the greater part of the posterior region of the body is removed at the same time the two branchial circlets begin to regenerate from the breaking joints as before but the opercular enlargements appear at the very beginning of regeneration. In this case an additional injury to the individual produces an acceleration of the rate of differentiation of the opercula.

The observations on the opercula of the Serpulid worms as well as simultaneous observations on the chelæ of the Crustacea

<sup>11</sup> Journal Exp. Zoöl., 1905, pp. 77-80.

mentioned below point to the view that additional injury in these cases at least results in an acceleration of the rate of regeneration from a particular level. They naturally led to a general investigation of the effect of the degree of injury upon the rate of regeneration.

### 3 The Arms of the Brittle-star,12 Ophioglypha lacertosa

A comparison was made of the rate of regeneration of individual arms from cuts at their bases when one, two, three, four and all five arms were removed. Care was taken to make the level of injury the same throughout. Nine individuals were used in each of the five groups and no food was given throughout the experiment. Measurements of the lengths of the regenerating arms were made 22, 33 and 46 days after the operation. The data are given in Table I and Figs. 2 and 3 of the Journal of Experimental Zoölogy, vol. ii, no. 1.

Notwithstanding the complications due to the age factor and the large degree of individual variation it is seen that there is an increase in the rate of regeneration of an arm as we pass from the cases with a smaller to those with a greater number of removed arms. The series with all five arms removed is excepted in this statement because the animals in this lot in every instance died or showed evidences of decay before the completion of the experiment. The difference between the series with four and that with only one arm removed is very evident. The intermediate cases with two and three arms removed show the same trend though somewhat obscured by individual variation.

The general results obtained with the arms of the brittle-star therefore confirm the suggestion given by the opercula of the Serpulids that an increase in degree of injury to the individual leads to an increase in rate of regeneration from a particular level. The results suggest also that this is not true indefinitely but that beyond a certain optimum degree of injury further injury is followed by a decline in the rate. When all five arms are removed

<sup>&</sup>lt;sup>12</sup> Biological Bulletin, 1903, and Journal Exp. Zoöl., 1905, pp. 7-77.

at their bases the degree of injury is evidently beyond the optimum. A point has been reached at which the injury is rapidly or gradually fatal in all cases and the rate of regeneration in those surviving for a time is slow. The rate of regeneration thus increases up to the optimum which is reached when four arms are removed. Beyond the optimum the rate slows down.

### 4 The Oral Arms of Cassiopea xamachana13

The rate of regeneration of a single oral arm in each of the following cases was determined:

- a One arm removed at its base.
- b Two arms removed at their bases.
- c Four arms removed at their bases.
- d Six arms removed at their bases.
- e All eight arms removed at their bases.

In the last four cases the averages of the rates of regeneration of the individual arms were taken. As in the case of the arms of the brittle-star there is a complication due to the age or size factor and to individual variation. The general result is however clear that there is an increase in rate of regeneration up to an optimum degree of injury beyond which further injury causes a decrease in rate. In this experiment the removal of four to six arms seems to constitute the most favorable degree of injury. When four arms are removed the rate is not as great as with six removed but the difference is so small that it may not be significant. On both sides of the optimum of four or six arms however there is a marked decline. The average specific amount of regeneration, i. e., the rate per unit of disk diameter, was 0.058 for a single removed arm, 0.112 for each of two removed arms, 0.144 for each of four removed arms, 0.157 for each of six, and 0.117 for each of eight. The data showing the considerable individual variation are given in Table I of the paper on regeneration in Cassiopea (Journal of Exp. Zoöl., vol. v, no. 2, 1907, p. 267).

<sup>18</sup> Journal Exp. Zoöl., 1907.

# 5 Larvæ of Amblystoma opacum. Nos. 2000–2031

A mass of eggs with the adult coiled over them was collected on October 1, 1906. The hatched salamanders were put into individual dishes about December 15 to avoid cannibalism. Four kinds of operations were made on December 15 with eight individuals for each kind. The essential precautions to avoid error as described on pp. 514 to 518 were taken. All individuals were killed in Gilson's fluid on January 8, twenty-four days after the operation. The four kinds of operation were:

- a Right fore-leg removed at its base.
- b All four legs removed.
- c Tail alone removed.
- d Tail plus all legs removed.

The data are given in Tables I and II. The lengths are in millimeters and the tail length equals the distance from the level of the hind legs to the tip of the tail.

Two kinds of comparisons may be made from the data:

- a The length of the regenerated tail when it alone is removed may be compared with the same when there is an additional injury involving the removal of the four legs. The specific amount regenerated is gives the best basis for a comparison. Seven individuals in each group are available and are arranged in order of their specific amounts in Table 3. Notwithstanding an overlapping in individual cases it is evident that on the whole the faster regeneration of the tail takes place when there is an additional injury involving all four legs.
- b The regenerated right foreleg when it alone is removed may be compared with the same when all four legs or the tail plus all four legs are removed. Unfortunately it is not possible on account of the various bends in the legs to make an accurate measurement. A general side-by-side comparison of the right forelegs in the sets with the three different degrees of injury did not reveal any differences of sufficient degree to be readily

<sup>&</sup>lt;sup>14</sup> The specific amount is the regenerated length divided by the removed length. The specific rate is the rate per unit of removed length per day.

	ECTODERM	NOTOCHORD	NEURAL TUBE	MUSCULAR TISSUE REGION	FIN REGION
3 days	Three irregular layers of cubical cells with mitotic figures.	-	Extending halfway to the tip of the regenerating tail. Relatively large.		Filled with closely packed round or cubical cells with large nuclei. No tissue of fibrous appearance
5 days	Three layers more regular.  The outer one relatively thinner, of somewhat flat-tened cells.		Same as three days.	Muscle fibers absent; a very few small sarcoblasts on and very near the level of injury.	before. Some cells with
7 days	Three layers distinct, cells of the outermost flat, those of the innermost columnar.	Wall very thin, composed	but relatively much longer.	A layer of sarcoblasts under the ectoderm in the muscle region.	Fins filled with loose fibrous tissue. Nuclei few ex- cept between the muscle regions.
to days	The same as at seven days.	Core an open reticulum with nucleated cells in a layer lining the wall. Wall thicker and without nucleated cells. 3-5 layers of connective tissue, with nuclei, drawn very closely around the wall.	Relatively smaller.  ——  Dorsal sorta definable.	Many of the sarcoblasts dif- ferentiated into small mus- cle fibers that take an eosin stain, and are grouped like the fibers of the old muscles. Many sarco- blasts still present among the developing-fibers.	out. Nuclei few except in region of the notochord. Connective tissue between the muscle regions form- ing a vertical partition.
12 days	Middle layer less evident. The inner and outer layers quite regular.	thicker. 3-5 layers of	Dorsal aorta with a thicker wall.	Muscle fibers larger; new groups of fibers appearing.	
14 days	The same as at twelve days.	days are continued.	No change in neural tube.  Dorsal aorta extending relatively nearer the tip of the new tail.	twelfth day are continued.	

### EXPLANATION OF CHART

The changes in state of differentiation of ectoderm, notochord, neural tube, muscle tissue, and connective tissue are written in vertical columns and correspond horizontally with the number of days after operation named in the column to the left.



TABLE 1

Amblystoma opacum. Nos. 2000-2031

	CATALOGUE		ORIGINAL MEASUREMENTS IN MMS., DECEMBER I					
CHARACTER OF OPERATION	NUMBER	Total body length	Total tail length	Length of re				
,			,					
	2002	33.1	16.2					
	2006	35.1	16.5					
	2010	31.2	13.8					
Right fore leg	2015	30.0	13.2					
	202 [	37.2	18.1					
	2024	33.0	15.3					
	2025	35.0	15.7					
Ų	2027	37.2	17.6					
ſ	2000	35.0	16 7					
	2001	35.2	16.3					
	2005	37 - 4	17.7					
All legs	2011	33.7	15.4					
nui iegs	2019	31.2	13.5					
	2022	29.2	13.7					
	2023	33.0	15.2					
	2029	37.0	17.5	1				
(	2003	31 8	15.2	12.3				
1:	2007	35.8	17.0	12.2				
ļ.	2012	35.3	17.1	13.7				
	2013	32.7	14.7	12.2				
<b>Γa</b> ıl	2017	38.0	19.0	15.6				
	2018	35.0	16.8	13.7				
	2020	28.9	13.0	9.7				
l'	2030	34.1	15.3	13.3				
·			-1 -	_				
'	2004	34.2	16.0	14.4				
<b>'</b>	2008	31.8	15.2	12.4				
$\downarrow_{\parallel}$	2009	34.7	15.9	12.9				
Fail plus all legs	2014	35.6	17.3	12 8				
	2016	28.2	12.0	9.2				
	2026	38.1	18.0					
	2028	35.6	16.5	12.9				
U.	2031	32.0	15.1	13.0				

TABLE 2

Amblystoma opacum. Nos. 2000-2031

		FINAL MEAS	UREMENTS.	PRESERVED ANI	IMALS, JANU	ARY 8, 19
CHARACTER	CATALOGI F	Total	Total	Length		
OF OPPRATION	NUMBER	body	tail	of regenerat-	Specific	Specific
i		1		ing tail	amount	rate
		length	length	ing tan		
ſ	2002	34.4	16.3			
light fore leg	2006	35.8	16.8	i i		
	2010	32.9	14.5			
Right fore leg.	2015	30.7	13.7			
	2021	33.0	14.4			
1	2024	30.5	13.9	i		1
Į.	2025	31.5	13.7			
ì	2027	33 -5	15.5			} !
ſi	2000	36.2	16.9			
All legs	2001	35.6	16.8			i i
	2005	36.0	16.7			1
	2011	34.6	16.0			l
	2019	31.1	13.6			!
	2022	29.1	13.2	1		
li	2023	32.6	14.7		,	
Ü	2029	33.8	14.8		•	
(	2003	!				
['	2007	29.2	10.3	4.5	0.37	0.015
	2012	28.9	10.2	5.2	0.38	0.016
Cail	2013	27.4	9.8	4.9	0.40	0.017
1	2017	29.0	10.1	4.5	0.22	0.009
	2018	28.5	10.0	4.9	0.36	0.015
j	2020	25.0	9.6	4.9	0.51	0.021
Ų	2030	24.4	7.9	4.6	0.35	0.015
(	2004	29.3	9.3	5.0	0.35	0.015
11	2008	28.1	10.3	5.9	0.48	0.026
	2009	28.8	10.2	5.8	0.45	0.019
ail plus all	2014	28.0	10.5	5.7	0.45	0.019
legs	2016	25.0	9.3	5.2	0.57	0.024
0	2026	28.8	9.9	5.0	<i>3</i> ,	
	2028	29.7	11.1	4.9	0.38	0.016
11	2031	26.5	9.1	5.3	0.41	0.017

TABLE 3

Amblystoma opacum. Nos. 2000–2031. Individuals arranged in order of specific amounts of tail regeneration, twenty-four days after the operation

TAIL ALONE	TAIL PLUS
RFMOVED	ALL LFGS
	REMOVED
0.22	
0.35	0 35
0.36	
0 37	
0 38	0.38
0.40	
ď	0.41
	•
	0.45
	0.45
	0.48
0.51	
•	0 57
	, 0 3/

noticeable. The numbers of compared individuals in the three sets are eight, eight, and seven respectively beginning with the lowest injury.

The following four experiments on Amblystoma (Nos. 6, 7, 8 and 9) were planned only partly with regard to the question of the effect of degree of injury on the rate of regeneration. They give some data on the present problem, though in most cases the comparisons are merely between a very few individuals.

# 6 Tail of the Larva of Amblystoma opacum. Nos. 967-990

The rate of regeneration of the tail when it alone was removed was compared with the rate when one or both of the fore-legs were removed at the same time.

From a mass of eggs belonging to a single salamander collected on October 1, 1905, the young were hatched on October 4.

Twelve of the thirty larvæ are available for present purposes. Four groups of three individuals each were operated upon November 8 and 9 and all the individuals were killed seventeen days

after the operation. The necessary precautions employed are given in the general outline of the sources of error (pp. 514 to to 518). In each group there is an individual with the tail alone cut off, one with the tail plus the right fore-leg and one with the tail plus both fore-legs removed. The lengths of the regenerated tails and other measurements are given in Table 4. With the exception of one individual (No. 969) it is seen that the per cent

TABLE 4

Amblystoma opacum

T - Tail alone removed
T + r. F. L. - Tail plus right fore-leg removed
T + b. F. L. - Tail plus both fore-legs removed

	CATALOGUE NUMBER	PARTS REMOVED	TOTAL ORIGINAL BODY LENGTH IN MMS.	REMOVED PART OF TAIL IN MMS.	LENGTH OF REGEN- ERATED TAIL IN MMS.	PER CENT REGENER- ATED
	967	T	25.2	6.2	2.6	42
Group one	968	T + r. F. L.	-	?	3.1	50+
	969	T + b. F. L	23.9	5.9	2.0	34
	974	T .	20.1	6.0	3.7	62
Group two {	975	T + r. F. L.	20.4	5.8	3.8	66
į	976	T + b. F. L.	20.0	5.1	3.7	73
1	981	T	22.3	6.6	3.0	45
Group three $\left.\left.\left.\left.\left.\right.\right.\right.\right.\right.$	982	T + r. F. L.	21.9	, 6.o	3.1	52
l	983	T + b. F. L.	20.9	5.2	3.6	69
(	988	т	22.3	6.6	2.2	33
Group four {	989	T + r. F. L.	22.0	6.6	2.4	36
-	990	T + b. F. L.	21.7	5.5	2.4	44

of the removed length of the tail regenerated is least in the individuals with the tail alone removed and increases with increase in additional injury.

The special objection to the results of this experiment applies equally well to the following observations on Amblystoma. These observations were made as parts of a larger experiment and in all only a few individuals are available for comparison. The

our groups in the present case should be considered separately since no special attempt was made to keep the conditions alike throughout all four.

# 7 The Right Fore-leg of the Larva of Amblystoma opacum Nos. 1026–1034

The size and stage of differentiation of the regenerating right fore-leg in individuals with no additional injury were compared with the regenerating right fore-leg in cases in which the other three legs and the tail were removed at the same time. Nine individuals were used. These came from a set of eggs collected on October 1, 1905, and hatched on October 4. The operations were made on February 7. The animals were killed in 85 per cent alcohol on March 17, thirty-eight days after the operation. Only three individuals are valid for the comparison. In individual No. 1033 with the tail and all four legs removed each regenerating fore-leg is larger and its toes are longer and more developed than in the right fore-legs of individuals Nos. 1027 and 1030, in which the right fore-legs alone were removed. The character of the regenerating organ makes it impossible to give exact measurements. However there is no doubt that each of the two individuals with the lesser degree of injury has a smaller and less differentiated right fore-leg than the one with an additional injury involving the other three legs and the tail.

# 8 Larvæ of Amblystoma opacum. Nos. 1035–1046

As before, all the larvæ are from a single egg mass. They were collected October 1, 1905, and hatched October 10. The operations came on February 8 except Nos. 1043 and 1044, which were made on February 9. All were killed on March 17, thirty-six or thirty-seven days after the operation. Three separate comparisons on the effect of degree of injury are possible.

a The size and stage of development of the right fore-leg was compared in the cases with different degrees of injury. In individuals Nos. 1040 and 1044 the right fore-leg alone was removed. In Nos. 1035, 1042 and 1046 the right fore-leg plus

the other three legs and the tail were removed. No differences in size of the regenerated right fore-leg were apparent. The toes however showed differences in degree of development.

In Nos. 1035 and 1042 the fourth toe was well developed.

In Nos. 1044 15 and 1046 the fourth toe was beginning to develop. In No. 1040 there was no fourth toe.

Evidently the fourth toe on the whole appears earlier in the individuals with a greater injury than in those with a lesser injury. In this group there is no apparent difference in size of the regenerating organs but an evident difference in the stage of differentiation.

b The size and stage of development of the right hind-leg were compared in cases with different degrees of injury. In Nos. 1037, 1039 and 1043 the right hind-leg alone was removed.

In Nos. 1035, 1042 and 1046 the right hind-leg plus the three other legs and the tail were removed. No difference in the size or stage of development of the regenerated right hind-legs could be made out.

In this group as far as could be seen additional injury neither retarded nor accelerated the rate of regeneration.

c The length of the regenerated tail in individuals in which the tail alone was removed was compared with that of individuals in which there was an additional removal of all four legs. The lengths of the regenerated tail in the three individuals with the lesser injury were 2.6, 2.9, and 2.9 mm. respectively. In those with the greater injury, 2.4, 2.5 and 2.6 mm. This is an advantage in favor of the lesser injury.

Salamanders Nos. 1035–1046 thus show a more rapid differentiation of the right fore-legs and a less rapid growth of the tail in the cases with a greater injury.

### 9 Larvæ of Amblystoma opacum. Nos. 1047-1057

All these salamanders came from one egg mass collected on October 1, 1905, and hatched on October 10. The operations were made on February 8 and the animals were killed on May

<sup>15</sup> The regenerating foot is double.

17, thirty-seven days after the operation. Three sets of comparisons similar to those of Experiment 8 may be made.

- a The regeneration of the right fore-leg when it alone was removed was compared with the regeneration of the same organ:
- (1) when the other three legs were removed at the same time and (2) when the other three legs plus the tail were removed. Two

individuals are valid for comparison in each case.

The two groups with the greater injury were alike in size of the legs and stage of development of the toes. When compared with the one individual in which the right fore-leg alone was removed all four individuals with additional organs removed have a larger right foreleg but there is no difference in the stage of development of the toes. Difference when present is here in favor of the greater injury, though this comparison by itself would have but little weight.

- b The regenerated right hind-leg (No. 1051) and the regenerated left hind-leg (No. 1057), one leg alone being removed in each case, were compared with the regenerated right or left hind-legs in cases with an additional injury involving
  - 1 All four legs (Nos. 1048 and 1054).
  - 2 Tail plus all four legs (Nos. 1049 and 1055).

In stage of development of the toes these six individuals can be arranged as follows beginning with the one having the greatest development:

1049, 1048, 1055 = 1054 = 1051, 1057. Individuals with an additional injury are evidently ahead of those with no additional injury. On the whole also the ones with the greater additional injury have an advantage over those with a lesser additional injury.

c One individual with the tail alone removed had regenerated 2.8 mm. of its tail, while of the two individuals with an additional removal of the four legs, one had regenerated an equal amount (2.8 mm.) and the other only 2.3 mm. While insufficient for a definite conclusion the data favor the lesser degree of injury.

Taking the five sets of experiments on salamander larvæ together the results on the whole evidently favor the individuals with additional injury.

## 10 The Tail in Tadpoles of the Green Frog, Rana clamitans Nos. 911–962

The length of the regenerated tail in individuals with that organ alone removed was compared with the length in others with an additional removal involving one or both hind-legs. Twenty-three of the fifty-six tadpoles give data that are valid for the present comparison. The operations were made on October 20 and 21, 1905; the first measurements of length of the regenerating tail on November 13 and 14, twenty-four days after the operation; the second measurements, January 27, ninety-nine or one hundred days after the operation and the third and final measurements from specimens killed on March 18, one hundred and forty-nine or one hundred and fifty days after the operation. The last were preserved in 85 per cent alcohol and the apparent decrease in the lengths as given is due to shrinkage caused by the alcohol.

The data showing the total lengths, the stage of development of the hind-legs, the tail lengths, the removed tail lengths, the amounts regenerated and the specific amounts of regeneration or the amounts per unit of removed length are given for each of the four measurements in Tables 5 and 6.

The tails were removed by a transverse cut near their middl and the hind-legs by cuts through the thighs. The catalogue numbers are given for comparison with the same individuals as used in other experiments.

The individual variations are so great that nothing more than a general determination of the effect of degree of injury to the individual can be made from the present data alone. The difference shown is on the whole without any doubt in favor of the tadpoles with an additional injury as opposed to those without such injury. The data are most complete at twenty-four days after the operation. If the individuals in each group are compared separately with regard to absolute amounts of regeneration it is found that in a majority of cases the length of the regenerating tail is greatest when the tail plus the left hind-leg is removed the tail plus both hind-legs coming next and the tail alone removed

TABLE 5

Tadpoles of Rana clamitans. Nos. 911-962

Tail removed

T + l. h. l = Tail plus left hind leg removed

T + b. h. l.'s = Tail plus both hind legs removed

Lengths are in mms.

Measurements at time of operation, October 20-21, 1905. Measurements, November 13-14, 24 days after operation

		-						-		~	
CHARACTER OF OPERATION	CATALOGUE NUMBER	TOTAL LENGTH	HIND LEGS ANGLE AT KNEE JOINT	TOTAL TAIL LENGTH	LENGTH OF PORTION OF TAIL REMOVED	TOTAL LENGTH	HIND Right	Left	TOTAL TAIL LENGTH	LENGTH OF REGENERAT- ING PORTION OF TAIL	SPECIFIC AMOUNT REGENERATED
****			degree				degree	degree			•
T + b.h.l.s.	110	63.5	100	39.4	21.0	52 2		reg. knob	29.7*	11.0*	0.52
T + l.h.l .	912	64.8	obtuse	41.4	23.3	55.4	100		34.8	12.8	0.55
т	913	59.5	obtuse	38.2	22.2	0.17	135	135	30.8	12.0	0.54
T + b.h.l's.	918	60.6	100	37.5	19.3	54.1			33.3	11.6	0.60
Т	920	58.8	130	36.0	21.7	50.8	90	90	29.1	10.4	0.48
T + b. h. l's.	925	70.9	85	47.0	21.5	60.0	•		37.5	9.5	0.44
$T + l. h. l \dots$	926	72.8	80	48.2	23.0	61.1	80		37.9	12.8	0.56
т	927	65.6	120	43.0	22.5	52.2	130	130	30.3		0.42
T + b. h. l's	932	72.2	95	48.0	23.0	58.6	ŭ	1	37.0		0.43
T + l.h.1	933	69.8	90	45.7	22.5	57 - 5	90	•	35.6	10.8	0.48
T	934	75.5	80	50.6	22.0	62.4	60	60	39.4	10 4	0.47
T + b. h. l's	939	66.3	95	43.6	20.0	56.6		1	35.7	11.3	0.56
T + 1. h. 1	940	69.0	110	47.3	21.4		100	reg. knob	37.2	10 1	0.47
T	941	69.9	110	46.4	19.5	58.8	125	125	38.8	9.0	0.46
T + b. h. l's	946	65.3	100	45.0	22.5	53.9	•		31.9	9.3	0.41
$T + l. h. l. \dots$	947	73.0	110	50.3	23.2	58.2	110	small knob	38.0	9.0	0.39
T	948	70.3	80	48.6	24.0	55.5	• 90	90	34.7	9.3	0.39
T + b. h. l's	953	65.6	95	42.1	19.7	52.1			30.4	7.3	0.37
T + 1. h.1	954	62.7	150	39.9	17.7	51.0	135	small knob	30.2	8.6	0.49
T		71.2	85 -	47 - 5	25.7	51.6	90	90	31.1	7.6	0.30
T + b. h. ls	960 '	62.1	150	41.8	18.0	48.9	reg. knob	:	30.3		0.38
T + l.h.l	961 j	64.0	150	43.0	22.2	51.0	135	· ,	30.6	8.5	0.38
T	962	58.9	150	39.5	19.0	46.7	135	135	28.6	8.1	0.43
		!	<u> </u>		1						

TABLE 6

Tadpoles of Rana clamitans. Nos. 911-962

Measurements January 27, Individuals killed, March 18, 99-100 days after the operation 149-150 days after the operation HIND LFGS LENGTH OF REGENER-TOTAL REGENERATED HIND LEGS CATALOGUE NUMBER TOTAL TAIL LENGTH TOTAL TAIL LENGTH CHARACTER OTAL LENGTH OF OPERATION Right dega. degs. degs. reg. bud 31.4 14 7 0.70 T + b. h. l's. . 911, 55.3 reg. bud 33.6 13.9 0.66 51.5 T + 1. h. 1. . . 912 57.9 38.5 15.8, 0.68 ..... 913 55.3 135 35.5 15.6 0.70 135 T + b. h. l's. 918 57.0 37.0 17 0 0.88 T ..... . 920 55.9 35.3 17.5 0.81 50.2 110 100 30.3, 16.0, 0.74 T + b. h. l's. 925 60.0 39.0 12.2 0.57 50.4 31.7 10.7 0.50 T..... 927 53.7 135 34.1 13.5 0.60 45.5 100 | 26.9 9.6 0.43 135 100 T + 1. h. l... 933 58.2 38.2 12.7 0.57 T + b. h. Ps. 939 58.6 37.2 13.0 0.65 54.9 33.7, 12.5, 0.622 T + l. h. l...38.0 13.0 0.61 940 57.2 120 small reg. bud T..... 941, 57.4 120 120 38.8 9.1 0.47 49.5 110 110 8.8 0.45 T + b. h. l's. 946 53.5 33.2 11.4 0.51 T + 1. h. 1... 947 59.0 small reg. 38.6 10.3 0.44 bud T. .... 948 56.4 80 80 36.4 11.9 0.50 46.9 90 ! 29.0 9.6 0.40 T + b. h. l's. 953 51.2 32.0 10.7 0.54

coming last. The same is true when the specific amounts of regeneration are compared. The comparison of individuals within a group is more accurate than an inter-group comparison because the control of the factors involved in general treatment of the animals is better. The dishes within a group were always kept together and operations were made at the same time. However, if all the individuals of the experiment are taken without regard to group boundaries the same general result is obtained. The animals with an additional injury of one hind-leg show the greatest average amount of regeneration of the tail, those with both hind-legs removed nearly as great an average amount, while those with the tail alone removed show the least tail regeneration. The average specific amounts are 0.487, 0.464 and 0.436 respectively.

The later measurements suffer considerably from the large number of deaths, the series with one hind-leg removed suffering more than the others. Ninety-nine or one hundred days after the operation the average specific amounts are 0.623 for the greatest injury, 0.572 for the medium injury and 0.616 for the tail alone removed. One hundred and forty-nine or 150 days after the operation all those with the medium injury had died. The ones with an additional injury of both hind-legs show an average specific amount of regeneration of the tail equal to 0.605 as opposed to 0.505 for those with the tail alone removed. The apparent shrinkage in the last measurements is not real but due to preservation in alcohol, the other measurements having been made from the living animals.

The specific amounts of regeneration are given in Table 7. The individuals are arranged according to magnitude of specific amount.

# Summary of Experiments on Animals Without a Molting Habit

A general survey of the foregoing experiments on animals without a molting habit may be profitable. All my experiments that give any data on the problem are included. Taken as a whole the data are favorable to the view that within moderate

TABLE 7

Tadpoles of Rana clamitans. Nos. 911-962. Specific Amounts of Tail Regeneration

TWENTY-FOUR DA	OPERATION .		NE TO ONE	HUNDRED	TO ONE-H	E HUNDRED AND FORTY-NINE O ONE-HUNDRED AND FIFTY- AYS AFTER THE OPERATION		
Tail + both hind legs	Tail + left hind leg	Tail alone	Tail + both hind legs	Tail + left hind leg	Tail alone	Tail + both hind legs	Tail + left hind leg	Tail alone
0.37		0.30	0.51	0.44	0.47	0.50		0.40
0.38	0.39	0.39	0.54	0.56	0.50	0.625		0.43
0.41	0.47	0.42	0.57	0.61	0.60	0.70		0.45
0.43	0.47	0.43	c.65	0.68	0.70			0.74
0.44	0.48	0.46	0.66		0.81			
0.52	0.49	0.47	0.81		l	. :		
0.565	0.55	0.48				'		
0.60	0.56	0.54						
Av0.464	0.487	0.436	0.623	0.572	0.616	0.608		0.505

degrees of injury an additional injury to an individual increases rather than decreases the rate of regeneration of a part. These data are based on experiments with several widely separated groups of animals. They support the general proposition that when an organ involving only a moderate disturbance of an animal's activity is removed, its rate of regeneration is less than it is in the case the individual is injured to a moderate degree in other parts at the same time. In general terms the rate increases up to an optimum degree of injury to the individual; it then remains stationary, and with still greater injury it decreases.

#### EXPERIMENTS ON ANIMALS WITH A MOLTING HABIT

Animals with a hard cast that has to be shed at intervals have an obvious disadvantage for the present purpose as compared with non-molting animals. It has been shown that the relation of the time of the operation to the time of the molt has a very great influence upon the amount of regeneration taking place in a given time. Likewise there is a variation in the rate of regeneration with the length of the molting period. A study of the problem has revealed a very complicated interrelation of age molting time, degree of injury, rate of growth and rate of regeneration. On the whole the specific amount of regeneration per molting period as a unit is more constant than the amount per day, other factors being alike. For this reason in the majority of the experiments the molting period is used as the unit.<sup>16</sup>

Notwithstanding their molting habit Crustacea have some very distinct advantages in a study of the problem in hand which partly offsets the disadvantages. The presence of a definite breaking joint in the chelæ makes it possible to be sure of the constancy of the level of regeneration. Furthermore Crustacea can be readily preserved after the completion of an experiment without danger of shrinkage and measurements can be more accurately made on them than on living animals.

# I The Fiddler-crab, Gelasimus pugilator17

In the male fiddler-crab one of the two chelæ is much larger than the other. In the female both are small and of equal size. The following plan of experiment was followed:

# A Experiments on Males

- Set a Large chela alone removed. Twenty individuals were kept for sixty-two days.
- Set b Small chela alone removed. Ten individuals were kept for sixty-two days.
- Set c Both chelæ removed. Ten individuals were kept for sixty-two days and eighteen others for forty-two days.

# B Experiments on Females

- Set a One chela was removed in six individuals.
- Set b Both chelæ were removed in three individuals.

<sup>&</sup>lt;sup>16</sup> See paper on Successive Regeneration. This journal, vol. vii, no. 3.

<sup>17</sup> Journal of Exp. Zoöl., vol. ii, no. 1, p. 81.

It was found that the crabs with both chelæ removed molted sooner than those with only one chela removed. The regenerating buds were in general larger in the specimens with two removed chelæ than in those with one removed. This was true of both molted and non-molted individuals.

# 12 The Chelæ of Alpheus dentipes18

In Alpheus dentipes the two chelæ have different functions. One is larger than the other and different in structure. The larger is called the "snapping" chela, the smaller the "cutting" chela. In nature either one may be on the right side. When the cutting chela alone is removed a new cutting chela grows out in its place. When the snapping chela is removed the uninjured cutting chela is transformed into a new snapping chela while in place of the removed snapping chela a new cutting chela develops. Finally, when both chelæ are simultaneously removed a new snapping chela grows out in place of the old snapping and a new cutting in place of the old cutting chela. The gradation in degree of injury to the individual as a whole beginning with the lowest injury is,

- I Cutting chela alone removed.
- 2 Snapping chela alone removed.
- 3 Both chelæ removed.

In each case a cutting chela is developed, in the first two cases from the single cut surface and in the last case from one of the two cut surfaces.

The rate of molting increases with increase in degree of injury. The average molting period for the set with the lowest injury was 29.6 days, for the next 28.7 days and for the set with the greatest injury 22.9 days.

Notwithstanding the shorter absolute time allowed to the series with the greater injuries the lengths of regenerating cutting chelæ<sup>10</sup> were greater in these than in the series with the lesser

<sup>18</sup> Journal of Exp. Zoöl., vol. ii, no. 1, p. 85.

<sup>&</sup>quot;'Cutting chelæ" refers to resultant organs.

injuries. Thus at the end of the second molting period the series with the least injury had regenerated 74.3 per cent of the cutting chela length, the second series 75.1 per cent and the series with the greatest injury 87.0 per cent. The difference between the last and first two is striking especially in view of the difference in molting times.

# 13 The Chelæ of the Crayfish, Cambarus propinquus20

The chelæ are approximately equal in size. A comparison of the rate of regeneration of a chela in individuals with it alone removed was made with its rate in individuals in which both clelæ and the last two pairs of walking legs were removed. Seventy-seven mature cray-fish ranging in cephalo-thoracic lengths from 10 to 20 mm. were used. Thirty-six of these had the lesser and forty-one the greater injury. The data are given in the Journal of Experimental Zoölogy, vol. ii, no, 3, pp. 350 ffl.

It was found that the crayfish with the greater injury molted more rapidly than the ones with the lesser injury. The amount of regeneration at the end of a molt was approximately equal in the two series, but because of the acceleration in the molting time the actual rate was greater in the series with the greater injury. Thus among the males in the series with the lesser injury (fourteen available individuals) the specific rate of regeneration<sup>21</sup> of the right removed chela was  $0.0049 \pm 0.0003$  against  $0.0080 \pm 0.0005$  for the same chela in individuals with the additional injury (thirteen cases).

Among females the corresponding figures are  $0.0030 \pm 0.0001$  (fourteen cases) for the lesser injury and  $0.0083 \pm 0.0007$  (twenty cases) for the greater injury.

In each case the difference is very strikingly in favor of the greater injury notwithstanding the considerable variations among individuals.

<sup>20</sup> Journal of Exp. Zoöl., vol. ii, no. 3, p. 347.

<sup>21</sup> The specific rate of regeneration is the rate in mms. per unit of thoracic length per day.

# 14 The Chelæ of the Shrimp, Palæmonetes vulgaris (Stimp) Nos. 1806-1861<sup>22</sup>

The object of the experiment was the comparison of the rate of regeneration of a single chela when it alone is removed with the rate of the two chelæ when both are removed.

Fifty-six shrimps were collected at Wood's Hole on September 2, 1906. Eighteen of these were "control" specimens without an operation, twenty had both chelæ removed and eighteen had one chela removed, the right in nine and the left in the other nine. The operations were made on September 2 and all animals were killed on September 10 giving a regeneration interval of eight days.

Only sixteen out of the thirty-seven available operated shrimps molted during the eight days of the experiment. Eight of these had the greater and eight the lesser injury. All thirty-seven individuals are however available for the comparison of rates of regeneration because in Palæmonetes vulgaris the new chela grows out directly and is not curled up in a chitinous sac as in the crabs. It is therefore unnecessary to wait for the molt. As a matter of fact the data show that the molted individuals are not necessarily the ones with the greatest length of regenerating chelæ.

The data are given in Tables 8, 9, 10 and 11. Table 8 gives the data for the shrimps in which the left chela alone was removed. Table 9 gives the data for those in which the right chela alone was removed. Table 10 gives the corresponding data for the ones in which both chelæ were removed. In Table 11 the specific amounts of regeneration are compared. They are arranged in order of magnitude beginning with the lowest, except the left chela in shrimps with both chelæ removed and regenerating. In these last the left chelæ are put opposite the right ones of the same individuals. Since the period of regeneration is eight days in every case the specific rates are comparable in the same way.

In individuals with both chelæ removed (nineteen cases) the average specific amount of regeneration of the left chela is 0.282

<sup>&</sup>lt;sup>22</sup> I am indebted to the acting director of Wood's Holl Marine Biological Laboratory, Professor Frank R. Lillie, for the use of a room during August and September, 1906.

TABLE 8

Palæmonetes vulgaris. Nos. 1806–1861. Left chela alone removed. Animals killed eight days after the operation

		1	LEFT	CHELA	SPECIFIC AMOUNT
NUMBER -	THORACIC LENGTH	FINAL RIGHT	Original length	Regenerated length	OF REGENERATION
1810	9-3	2.85	2.88	0.62	0.22
1816	9.3	2.80	2.80	0.77	0.27
1822	11.6	3.89	3.68	0.62	0.17
1828	12.1	4.04	4.00	0.69	0.17
1834	8.4	2.70	2.64	1.10	0.42
1840	12.2	4-43	4.52	0.79	0.17
1846	11.1	3.81	3.81	0.82	0.22
1852	10.5	3.41	3.41	0.83	0.24
1858	9.1	2.65	2.66	0.73	0.27
verage					0.239

TABLE 9

Palæmonetes vulgaris. Nos. 1806-1861. Right chela alone removed. Animals killed eight days after the operation

	FINAL CEPHALO-		RIGH:	T CHELA	SPECIFIC
NUMBER	THORACIC LENGTH	FINAL LEFT CHELA LENGTH	Original length	Regenerated length	AMOUNT OF REGENERATION
1807	br	2.96	2.93	0.82	0.28
1813	6.1	2.00	88.1	0.60	0.32
1819	11.1	4.07	4.02	0.55	0.14
1825	) 10.0	3.11	3.04	0.70	) 0.23
1831	8.0	2.51	2.52	0.52	0.20
1837	9.7	2.95	2.95	0.79	0.27
1843	11.1	3.77	3 · 59	0.48	0.13
1849	9.1	2.74	2.62	0.52	0.20
1855	9.7	2.75	2.81	0.85	0.30
verage					.230

TABLE 10

Palæmonetes vulgaris. Nos. 1806–1861. Both chelæ removed. Animals killed eight days after the operation

CATALOGUE	FINAL CEPHALO-	_	LEFT CHELA		·	RIGHT CHELA	
NUMBER	THORACIC	Original	Regenerated	Specific	Original	Regener-	Specific
	LENGTH	length	length	amount	length	ated length	amoun
1806	8.3	2 48	0.79	0.32	2.48	0.80	0.32
1809	8.9	2.62	0.59*	0.23	2.62	0.59*	0.23
1812	65	1.93	0.76	0.39	1.93	0.76?	0.397
1815	br	2.74	0.76	0.28	2.69	0.80	0.30
1818	11.8	3.92	0.94	0.24	3.92	0.90	0.23
1821	10.0	3.40	1.41	0.41	3.40	1.37	0.40
1824	10.4	3.12	18.0	0.26	3 14	0.89	0.28
1827	11.9	3.55	0.62	0.18	3.55	0.56	0.16
1830	9.8	3 06	0.65	0.21	3.13	0.68	0.22
1833	9.2	2.89	0.52	0.18	2.90	0.52	0.18
1836	9.1	2.69	0.59	0.22	2.69	0.59	0.22
1839	12.7	3.96	0.77	0.19	3.96	0.77	0.19
1842	10.0	3.11	1.48	0.48	3.11	1.48	0.48
1845	12.1	4.24	0.84	0.20	4.24	0.93	0.22
1851	11.6	3.35	0.83	0.25	3 35	0.89	0.27
1854 -	8.4	2.56	0.74	0.29	2.56	0.74	0.29
1857	9.0	2.44	0.62	0.25	2.52	0.66	0.26
1860	7.9	2.37	1.26	0.53	2.37	1.36	0.57
1861	11.7	3.48	0.85	0.24	3.48	0.81	0.23
verage				0,282	l !		0.286

and of the right, 0.286. When the right chela alone is removed (nine cases) the average is 0.239. When the left chela alone is removed (nine cases) the average is 0.230.

The advantage is very evidently in favor of the shrimps with the greater injury. Though the individual data overlap, the difference in the average is great enough to be certainly significant.

# 15 Crabs. Nos. 594-601 Sp? and 602-614, Porcellana platycheles

In connection with experiments carried on at the Naples Zoölogical Station in 1902-1903 on the question of the reversal of asymmetry data were obtained on the effect of degree of injury

TABLE 11

Palæmonetes vulgaris. Nos 1806–1861. Specific amounts of regeneration of the chelæ arranged in order of magnitude

BOTH CHEL	AE REMOVED	LLFT CHFLA REMOVED	RIGHT CHELA
Left	Right •	Left	Right
0.18	0.16	0.17	0.13†
0.18	o 18†	0.17	0.14
0.19	0.19	0.17	0.20
0.22	0.22	0.22	0.20
0.20	0.22	0.22†	
0.21	0.22		•
0.23	0.23		0.23
0.24	0.23		!
0.24	0.23†	0.24	İ
0.25	0.26	0.27	,
0 25	0.27	0.27	0.27
0.26	0.28		0.28†
0.29	0.29	1	1
0.28	0.30		0.30
0.32	0.32†		0.32
0.39	0.39†	1	
0.41	0.40	0.42†	I
0.48	0.48†		<u> </u>
0.53	0.57†		İ
Avo.282	0.286	0.239	0.230

<sup>† =</sup> cases that moulted after the operation.

The individuals used were crabs of an undetermined species and others of Porcellana platycheles. They all showed asymmetry in the chelæ. The operations consisted of the removal (a) of the shorter chela alone, (b) the larger chela alone, and (c) both chelæ. The operations were made on January 16, 1903, and the animals were killed on March 8, fifty-one days after the operation. Several individuals did not molt. Others molted once and these are used in the following data. Table 12 gives the data for the crab of undetermined species and Table 13 for Porcellana platycheles.

TABLE 12
Chelæ of crabs, Nos. 594-601

PARTS REMOVED	CATA-	origi Rie	nal chi	FLA LEN		FINAL CHELA LENGTHS  Right Left				SPECIFIC LENGTHS REGENERATED	
	NO.	Length	Width	Length	Width.	Length	Width	Length	Width	Right	Left
Right .	594	49	3.3	4 9	2.5	3.4	1.8	4.7	2.4	0.69	
Right	598	3.5	1.4+	3 5	2 3	3.0	1.4	3.1	2.0	0.86	
Left	. 601	4.5	2.3	>	>	4 4	2.2	3.4	1.6		?
Both	596	36 1	2.5	4 1	2.1	30	1.4	2.9	1.3	0.83	0 71
Both	597	30	13+	2.5	19	2.2	1.0	2.2	1.0	0.73 ;	0.88
Both	599	29	τ3,	2.5	1.9	2 2	1.0	2.4	1.0	0.76	0.96

TABLE 13

Chelæ of Porcellana platycheles. Nos. 604-613

PARTS C	ATALOGUE	ORIGINAL LINGTHS		FINAL L	ENGTHS		AMOUNTS OF ERATION	
REMOVED	NO. 1	Right	Left	Right	Left	Right	Left	
Left	604	4.4	3.9	5.0	3 7 ?		0.95?	
Left	608	3.0	2.7	2.6	}			
Left	610	2.9	?	3.2	2.6		,	
Right	612	,	1.7	1.4	1.6		0.94	
Both	605	3.0	2.7	2.8	2.7	0.80	0.64	
Both .	613	1.6	1.9	1.7	1.5	1.06	0 79	

A comparison of the specific amounts of regeneration at the end of fifty-one days shows on the whole an advantage in favor of the individuals with the greater injury. In the undetermined species the specific amounts of regeneration of the shorter chela are 0.83, 0.88 and 0.96 respectively for the three individuals with both chelæ removed and 0.86 for the one individual with the shorter chela alone removed. In the case of the larger chela the specific amounts when both chelæ were removed are 0.71, 0.73 and 0.76 in the three individuals against 0.69 in the one individual with the longer chela alone removed.

In Porcellana platycheles the data are fragmentary, only three individuals being available. The specific amount of regeneration of the smaller chela when the larger is removed at the same time is 0.80 and 1.06 in the two individuals present against 0.94 in the one individual with the small chela alone removed. These data show no difference between the average of the two sets but no general conclusion can be drawn because of the small number of cases.

The data for both species as far as they show any difference with difference in degree of injury favor the increase in rate with increase in degree of injury.

# 16 The Chelæ of the Crayfish, Cambarus bartoni, Nos. 2218-2267

A comparison was made of the rate of regeneration of a single chela when it alone is removed with the rate of each of the two chelæ when both are removed. Fifty young crayfish from a single mother were used in the experiment. As soon as the young left the mother they were put into individual dishes and the treatment for all was as nearly alike as possible. The food consisted of Tubifex, a supply of the living worms being kept in each dish.

The mother crayfish with eggs was captured on December 14, 1906. The young were put into separate dishes on February 11, 1907. The data fall into five groups: (a) First regeneration in younger individuals.<sup>23</sup> (b) First regeneration in older individuals.<sup>24</sup> (c) Second regeneration. (d) Third regeneration. (e) Fourth regeneration,

## First Regeneration in Younger Individuals

The crayfish used were in the fourth molt at the beginning and in the sixth at the end of the experiment. Their lengths at the beginning were 6.0-6.5 mm. and at the end 7.7-8.3 mm. The operations were made two days after the fourth molt and the lengths of the regenerated chelæ at the end of the sixth molt were compared. The data are given in Tables 14 and 15.

<sup>23</sup> Used for third regenerations in the data on successive regenerations.

<sup>24</sup> Used for first regenerations in the data on successive regenerations.

TABLE 14

Cambaru bartoni. Nos. 2218-2267. Regeneration of a single removed chela. Younger crayfish.

Molts 4-6. The right chela was removed in all except No. 2242

CATAL-		INTERVAL	CEPH	LENGTHS	RACIC		LENGTH	LENGTH SPECIFIC OF REGEN- AMOUNT	
LOGUE NUMBER	IN DA	IN DAYS	Molt	Molt 5	Molt 6	LENGTH Molts 4-5	CHELA	OF REGEN- ERATION	REGENER ATION
2218	4-5-6	23	6.2	7.0		6.6	2.9	0.44	0.019
2219	4-5-6	26	6.2		8.2	6.6	3.5	0.53	0.020
2223	4-5-6	23	6.0		8.0	6.5	3.5	0.54	0.023
2227	4-5-6	20		7.3		6.8	3.6	0.53	0.0265
2238	4-5-6	23	6.3	7.4	8.1	6.8	3.6	0.53	0.023
2239	4-5-6	31	6.5	7.0	7.7	6.7	3.2	0.48	0.015
2248	4-5-6	24	6.4	7.0	i I	6.7	3.1	0.46	0.015
2252	4-5-6	28	6.4	7.2	8.2	6.8	3 - 5	0.51	0.018
2259	4-5-6	19	6.1	7.1	8.1	6.6	3.0	0.45	0.024
2262	4-5-6	19		7.0	8.3	6.5	2.9	0.45	0.024
2242	4-5-6	20	6.4	7 - 4		6.9	3.1	0.45	0.6225
Average.		23.3	6.28	7.16	8.09	6.68	3.26	0.488	0.0210

The molt numbers indicate the number of molts since hatching. There is a chance for error because the crayfish eat their casts. The specific amount is the amount regenerated per unit of cephalo-thoracic length. The specific rate is the specific amount per day. The "chela lengths" are the lengths of the propodites.

TABLE 15

Cambarus bartoni. Nos. 2218–2267. Regeneration of the chelæ when both are removed at one time.

Younger crayfish. Molts 4-6

CATA- LOGUE NUMBER	MOLTS	MOLTS	MOLTS	INTER-		alo-tho Length		AVER- AGF CEPHA-		H OF RE	GENER-	SPECIFIC AMOUNT OF	SPECIFIC RATE OF
		DAYS	Molt 4	Molt 5	Molt 6	RACIC LENGTH	Left	Right	Average	REGEN- ERATION	REGEN- ERATION		
2237 2245 2253	4-5-6 4-5-6 4-5-6	27 26 20	6.6 6.6 6.2	7.6 7.2 7.1	8.6 8.3 8.3	7.1 6.9 6.7	4.2 4.0 3.1	4.2 4.0 3.0	4.2 4 0 3.05	0.59 0.58 0.46	0.022 0.022 0.023		
Average		24.3	6.47	7.3	8.4	6.9	3 · 77	3 · 73	3.75	0.543	0.0223		

The average length of a single regenerated chela is 3.26 mm. for the eleven cases available as compared with a regenerated length of 3.77 mm. for the right and 3.73 mm. for the left chela in the three cases in which both chelæ were removed. The specific amounts of regeneration, in this case the amounts per unit of cephalo-thoracic length, are 0.49 for the single chela when it alone is removed and 0.54 for each of the two chelæ in individuals with both chelæ removed. The specific rates, i. e., the specific amounts per day are correspondingly 0.021 for the single chela and 0.0223 for each of the two chelæ. A regenerating chela in individuals in which additional regeneration is going on thus has an evident advantage over one in which no additional regeneration is present.

## First Regeneration in Older Individuals

The crayfish used were in the eighth to tenth molt at the time of the operation and regeneration continued through one molt. The operations were made two days after the molt. The cephalothoracic lengths range from 10.5 to 13.6 mm. The data are given in Tables 16 and 17.

TABLE 16

Cambarus bartons. Nos. 2218-2267. Regeneration of a single removed chela. Older crayfish. Molts
8-0 and 0-10

CATA-		INTERVALS	CEPHALO-	THORACIC	LENGTHS	LENGTH OF REGEN-	SPECIFIC AMOUNT OF	SPECIFIC RATE OF	
NUM- BER	MOLTS	IN DAYS	Molt 8	Molt 9	Molt 10	ERATING CHELA	REGENER- TION		
2220	9-10	43		11.9	13.0	6.0	0.50	0.012	
2221	9-10	27		11.7	12.1	5.4	0.46	0.017	
2241	8-9	27	10.5	11.3	1	5.3	0.50	0.019	
2246	8-9	30	10.5	11.7		5.7	0.54	810.0	
2257*	8-9	21*	12.04	12.9*		2.7*	0.225*	4110.0	
2267	8-9	20	?	11.3		4-4	0.42	0.021	
Average		. 29.4	10.5	11.58	12.55	5.36	0.484	0.017	

<sup>\*</sup>Not included in the averages because of extreme values in all measurements of length.

TABLE 17

Cambarus bartoni. Nos. 2218-2267. Regeneration of the chelæ when both are removed at one time.

Older crayfish. Molts 8-9, 9-10, and 10-11

CATA- LOGUE NUM- BER  INTER- WOLTS VAL IN DAYS	MOTME	1	СЕРНА	LO-THOR	ACIC LE	KGTH8	[	H OF RE		SPECIFIC AMOUNT OF RE-	SPECIFIC
	Molt 8	Molt 9	Molt 10	Molt 11	Left	Right	Aver- age	GENERA- ATION	REGEN- ERATION		
2222 2228 2233 2240 2258	9-10 8-9 8-9 8-9	27 22 30 20	10.9 br 10.5	11.9 11.3 11.6 11.9	12.1	13.6	5.4 5.1 6.8 4.1 5.9	5.5 5.0 6.6 4.5 6.0	5·45 5·05 6·7 4·3 5·95	0.50 0.48 0.55 0.41	0.019 0.022 0.018 0.0205 0.020
Average		24.8	10.7	11.67	12 55	13.6	5.46	5 52	5 · 49	0.488	0.020

The average lengths of the regenerated chelæ when a single chela is removed is 5.36 mm. for the five available cases as compared with a regenerated length of 5.46 mm. for the right chela and 5.52 mm. for the left chela in individuals in which both chelæ are removed. The average specific amount of a regenerating chela is 0.484 when it alone is removed and 0.488 when the other chela is removed at the same time. The average specific rate of a regenerating chela likewise is 0.017 when it alone i removed and 0.020 when the other chela is removed at the same time.

The advantage on the whole is evidently in favor of the regenerating chelæ in individuals with the greater injury.

### Second Regeneration

The data for second regenerations are given in Tables 18 and 19.

The average length of a regenerating chela in the nine cases in which it alone is removed is 4.5 mm. and in the three cases in which both chelæ are removed the averages are 5.1 mm. for the left chela and 5.1 mm. (?) for the right.

The average specific amount for the lesser injury to the individual is 0.521 and for the greater injury 0.563.

TABLE 18

Cambaius bartoni. Second regenerations. Single chela removed. Two molts

CATA-		TIME IN-	CFPHALO	-THORACIC	LENGTH8	AVERAGE CEPHALO-	LENGTH		SPECIFIC
NUM- BER	MOITS	TFRVAL IN DAYS	Molt 6	Molt 7	Molt 8	THORACIC LENGTHS Molts 6-7	OF REGEN- ERATEI CHELA	AMOUNT OF REGEN- ERATION	REGENER-
2218	6-7-8	24	br	9.3	10.1	8.8±	4.6	0.52	0.022
2219	6-7-8	. 32	8.2	9.2	9.9	8.7	4.2	0.48	0.015
2227	6-7-8	30	br	br	10.6	8.7土	5.1	0.59	0.020
2238	6-7-8	45	8.1	9.5	10.9	8.8	5.3	0.60	0.013
2239	6 7-8	50	7.7	8.2	8.7	7.95	3.7	0.47	0.009
2248	6-7-8	42	br	8.9	9.1	8.4±	3.7	0.44	0.010
2252	6-78	36	8.2	br	9.9	8.7±	4.6	0.53	0.015
2259	6-7-8	30	8 r	9.3	10.0	8.7	4.6	0.53	0.018
2262	6-7-8	29	8.3	9.3	10.4	8.8	4.6	0.52	810.0
2242	6-7-8	25	br	9.2	br	8.7土	4.6	0 53	0.021
Average		34 3	8.1	9.11	9.96	8.62	4.5	0.521	0.0161

TABLE 19

Cambarus bartons. Second regenerations. Both chelæ removed. Two molts

VEER		LENGTHS		CFPHALO-THORACIC		PHALO- ENGTH 5-7		IFNGTH OF REGENER-		INT OF	Jo N
CATALOGLE NO	MOITS		C CE	Left	Right	Average	SPECIFIC AMOUN REGENERATION	SPECIFIC RATE REGENERATIO			
2237	6-7-8	45	8.6	10.0	11.2	9.3	5.3	5.3	5.3	0.57	0.013
2245	6-7-8	38	8.3	9.3	10.5	8.8	5 4	*	5.47	0 61	0.016
2253	6-7-8	25	8.3	9.7	10.4	9.0	4 6	4.6	4.6	0.51	0.020
Average		36	8.43	9.67	10.7	9.03	5 1	5.17	5.1	0.563	0.0163

\*Chela deformed.

The average specific rate for the lesser injury is 0.0161 and for the greater injury 0.0163, a practical equality.

In the case of individuals with the greater injury a regenerating chela has the advantage in absolute amount of regeneration and in specific amount. In specific rate there is, however, a practical equality. In the present instance the specific amount makes a better basis for comparison not only for the general reasons given on pp. 534 and 535 but also because of the much greater uniformity of its values within a single group.

## Third Regeneration

The data for third regenerations are given in Tables 20 and 21 for cases extending over one molt only and in Tables 22 and 23 for cases extending over two molts.

In the one-molt cases the absolute amounts of regeneration are on the average 4.4 mm. in the case of removal of a single chela and 5.37 for each of the chelæ in case both are removed. The specific amounts are correspondingly 0.434 for the lesser injury and 0.50 for the greater injury and the specific rates 0.017 for

TABLE 20

Cambarus bartons. Third regeneration. Single chela removed. One molt

CATALOGUE	MOLTS	TIME INTER-			LENGTH OF	AMOUNT OF	RATE OF
NUMBER :		VAL IN DAYS	Molt 8	Molt 9	ATED CHELA	REGENERA- TION	REGENERA TION
2218	8-9	21	10.1	11.4	4.1	0.41	0.020
2219	8-9	23	9.9	11.2	2.7	0.27	0.012
2223	8-9	23	10.5	11.3	4.6	0.44	0.019
2227	8-9	31	10.6	12.1	5.4	0.51	0.016
2238	8-9	25	10.9	12.1	5.0	0.46	0.018
2239	8-9	20	8.7	9.4	2.8	0.32	0.016
2252	8-9	24	9.9	br	4.2	0.42	0.0175
2259	8-9	31	10.0	br	5.3	0.53	0.014
2242	89	33	br	11.6	5.4	0.55	0.017
verage		25.7	10.07	11.3	4.4	0.474	0.017

TABLE 21

Cambarus bartons. Third regeneration. Both chelæ removed. One molt

CATA- LOGUE NUM- BER	i	TIMF IN-	CEPHALO-T		LENGTH	OF REGEN	PERATED	OF REGEN-	RATE OF REGEN-
			Molt 8	Molt 9	Left	Right	Average		
2237	8-9	22	11.2	12 0	5 4	5.4	5.4	0.48	0.022
2245	8-9	26	10.5	11 8	5.3	*	5.3?	0.50	0.019
2253	8-9	30	10.4	11.5	5 4	5.4	5 4	0.52	0.017
Average.	1	26	10.7	11.77	5 · 37	5 37?	5 · 37	0.50	0.0193

\*Chela deformed.

TABLE 22

Cambarus bartons. Third regeneration. Single chela removed. Two molts

(ATALOGU) VUMBER		TIME IN-	(FPHALO-	THORACIC	LENGTH		LENGTH OF REGEN-	SPECIFIC -	
	MOLTS	DAYS	Molt 8	Molt 9	Molt 10	thoracic erated Lengths CHELA	OF REGEN- ERATION	REGEN- ERATION	
2218	8-9-10	41	10.1	11.4	12.6	10.75	6.0	0.56	0.014
2219	8-9-10	38	9.9	11.2	12.3	10.55	5.4	0.51	0.013
2223	8-9-10	54	10.5	11.3	12.6	10.9	6.4	0.59	0 011
2227	8-9-10	65	10.6	12.1	12.9	10.35	6.7	0.59	0.009
2248	8-9-10	35	9.1	10.2	11.0	9.65	4.9	0.51	0.015
2252	8-9-10	48	9.9	br	12.3	10.65	6.2	0.58	0.012
2259	8-9-10	68	10.0	br	12.0	10.5	6.3	0.60	0.009
2262	8-9-10	36	10.4	11.5	12.7	10.95	6.0	0.55	0.015
2242	8-9-10	71	br	11.6	13.0	105	6.7	0.64	0.009
Averag	ge	. 50.7	10.06	11.33	12.38	10.7	6 07	0.57	0.0119

the lesser injury and 0.0193 for the greater injury. The advantage is throughout in favor of the chela that has a regenerating mate.

In the two-molt cases the absolute amount of regeneration is on the average 6.07 mm. when a single chela is removed and 6.85 mm. for the left and 6.6 mm. for the right chela when both are removed. The specific amount is 0.57 for the single chela

TABLE 23

OGLE NUMBER	Can	TIME INTER- VAL IN		Thud re	-	VERAGE CEPHALO-	LENG	TH OF	Two molts  SPECIFIC  AMOUNT GF  REGENERA-  TION	SPECIFIC RATE OF REGENERA- TION	
CATAL		DAYS	Molt 8	Molt 9	Molt 10	Molts 8-9	Left	Right	Left Right	Left	Right
2245 2253	8-9-10 8-9-10		-			11.15			0.63 0 57	•	
Average		49.5	10.45	11 65	12 6	11.05	6 85	6.6	0.620 505	0.0125	0.012

and 0.62 and 0.595 for the left and right chelæ respectively in double removals. Likewise, the specific rate is 0.0119 for the single chela and 0.0125 and 0.0120 for the left and right chela respectively in double removals.

For the third regenerations again the advantage is in favor of the crayfish with the greater injury.

## Fourth Regeneration

Only four crayfish had a fourth regeneration. The regeneration in all extended through a single molt only. In three cases with a single chela removed the average absolute amount was 6.5 mm., the specific amount 0.52 and the specific rate 0.019. In the one case with both chelæ removed the average of the two chelæ gave an absolute amount of 5.5 mm., a specific amount of 0.46 and a specific rate of 0.021. The first two values are greater in the crayfish with the lesser injury, the last one is greater in the animal with the greater injury. The number of individuals is too small to make the result of much value.

Taking the present experiment as a whole it is very evident that in young crayfish the advantage is distinctly in favor of the chela with a regenerating mate, as opposed to a chela removed and regenerating alone.

## 17 Cambarus propinquus. Nos. 2309-2378

The present experiment and the following one (No. 18) were planned for a study of other factors than that of degree of injury. However, since they give some data on the problem in hand they are included.

The individuals in Experiment 17 come from a single mother collected with a mass of eggs in April, 1907. All the living young, seventy in number, were taken from the mother on May 12 and put into seventy separate dishes. All but seven of the crayfish had molted twice since hatching. The others molted their second time on May 13. The experiment was planned in part for a study of the effect of the time of the operation with respect to a molt upon the time of succeeding molts. Thirteen individuals are available for the purposes of the present paper. these were operated upon two days after the third molt. In seven one chela was removed and in six both chelæ were removed. In all except one individual in each set the molt came on May 21 and the operation on May 23. In the exceptional ones the molt came on May 20 in No. 2343 and on May 22 in No. 2358. The crayfish were killed in 85 per cent alcohol on June 20, twenty-eight days after the operation. The data are given in Tables 24, 25, 26 and 27. Tables 24 and 25 include the individuals in which three molts occurred during the regenerating period and Tables 26 and 27 those in which four molts occurred.

The young crayfish were so nearly equal in size that separate measurements of cephalo-thoracic length and removed chela lengths were not taken. It is, therefore, not possible as in the case of the data of the preceding experiment to give the specific amount and specific rate of regeneration. The absolute amounts and the rates per day are given in the tables. Of special value is the fact that the molting periods show only slight differences in length so that two compared groups are nearly equal in absolute time as well as in number of molts.

The data show an advantage in favor of the crayfish with two regenerating chelæ both in absolute amount and in rate per day For the three-molt cases the removed single chelæ have an abso-

TABLE 24

Cambarus propinquus. Nos. 2309-2378. One chela, the right, removed. Regeneration for three molts after the operation

CATALOGUE NUMBER	DAYS OF REGENERATION	LENGTH OF REGENER- ATED RIGHT CHELA IN MMS.	
2311	28	2.96	
2319	28	3.33	
2325	28	2.74	Rate per day = .110 mm.
2339	28	3.26	
verage	28.0	3.07	1

TABLE 25

Cambarus propinquus. Nos. 2309-2378. Both chelæ removed. Regeneration for three molts after the operation

CATALOGUE NUMBER	DAYS OF REGEN-	GENERALED	LENGTH OF RE- GENERATED RIGHT CHELA IN MMS.	
2320	28	3 · 33	3 · 33	Rate per day:
2341	29	3 - 55	3 · 55	Right chela = .114 mm.
2355	28	2.81	2.81	Left chela114 mm.
verage	28.3	3.23	3.23	

TABLE 26

Cambarus propinquus. Nos. 2309-2378. One chela, the right, Regeneration for four molts after the operation

CATALOGUE NUMBER	DAYS OF REGENERATION	LENGTH OF REGENER- ATED BIGHT CHELA	
<sup>2</sup> 354 <sup>2</sup> 358 <sup>2</sup> 364	27 28 28	3·77 3·33 4·14	Rate per day135 mm.
Average	27.7	3.75	

TABLE 27

Cambarus propinquus. Nos 2309-2378. Both chelæ removed. Regeneration for four molts after the operation

CATALOGUE NUMBER	DAYS OF	LENGTH OF REGENERATED LEFT CHELA	LENGTH OF REGENERATED RIGHT CHELA	
2317	28	3.92	4.00	Rate per day:
2327	28	3.88	3.88	Right chela = .140 mm.
2343	28	3.92	3.85	Left chela 140 mm.
Average	28.0	3 91	3 91	

lute amount of regeneration of 3.07 mm. and a rate per day of 0.110 mm. while the individuals with both chelæ removed have an absolute amount of regeneration of 3.23 mm. and a rate per day of 0.114 mm. in each of the two chelæ. For the four-molt cases the absolute amount for the single chelæ is 3.75 mm. against 3.91 mm. for each of the chelæ in the double removal and the rates per day are respectively 0.135 mm. and 0.140 mm.

# 18 Cambarus propinquus. Nos. 2391-2483

Ninety-three young of the one hundred and four attached to a single female were used in the experiment which was concerned primarily with other factors than the one discussed in the present paper. These young had molted twice since hatching and were put into individual dishes on May 22, 1907.

Those individuals which were operated upon one day after the third molt give data for the effect of the degree of injury Eight individuals with one chela removed and nine with both chelæ removed survived until June 20, when all were killed. A comparison is thus obtained between the rate of regeneration of a chela when it alone is removed and its rate when the opposite chela is removed at the same time.

The data are given in Tables 28 and 29. No measurements were made of cephalo-thoracic lengths or removed chela lengths and it is therefore not possible to make a comparison in terms of specific amounts and specific rates of regeneration.

TABLE 28

Cambarus propinquus. Nos. 2391-2483. One chela removed one day after the third molt

CATALOGUE NUMBER ; DA	YS OF REGENERATION	ERATED RIGHT CHELA	
2402	18	1.67	
2406	19	2.90	
2409	18	1.64	
2412	16	1.70	Rate of regeneration per day
2415	18	1.61	115 mm.
2421	18	2.37	
2435	17	i 2.13	
2477	17	2.20	•
Average	17.6	2.03	

TABLE 29

Cambarus propinquus. Nos. 2391-2483. Both chelæ removed one day after the third molt

CATALOGUE NUMBER	DAYS OF REGENER- ATION	LENGTH OF REGENERATED LEFT CHELA	LENGTH OF REGENERATED RIGHT CHELA	
2400	19	1.48	1.55	, 1
2410	18	1.82	1.82	· !
2424	17	1.44	1.44	
2426	19	1.82	1 52	Rate of regeneration per day:
2428	18	2.43	2.43	Left chela = .115 mm.
2437	19	3.10	3.10	Right chela113 mm.
2451	17	2.35	2.28	
2459	18	2.25	2.12	
2470	18	2.13	2.16	1
Average	18.1	2.09	2.05	

The average regenerated lengths are slightly greater in each of the two regenerated chelæ of a double removal than in the one chela of a single removal. The amounts are 2.09 mm. and 2.05 mm. for the double removal and 2.03 for the single removal. The rate per day is the same for one of the two chelæ of a double removal as for the single chela of a single removal. The other

chela of the double removal is slightly smaller on the average. The rates are 0.115 mm. and 0.113 mm. per day for the double and 0.115 mm. for the single removal.

The individual variations in the present experiment are considerable. The differences between the two degrees of injury are so slight that they are probably not significant. No change in rate of regeneration with degree of injury is indicated in the individuals of the present experiment.

#### DISCUSSION

The various factors controlling growth and regeneration are so closely interrelated that no final analysis of any one can be expected until there is at least some understanding of all or nearly all of them. This is nowhere more evident than in the case of the rate of regeneration.

Nevertheless the data collected in the preceding experiments point conclusively to the general fact that the rate of regeneration of a part does not necessarily diminish with additional injury to the individual. On the contrary in the majority of the cases the rate of regeneration of a part is evidently higher when other parts of the animal have been removed at the same time than when the part alone has been removed.

It is evident that the amount of injury involved in the removal of the part whose rate of regeneration is being compared as well as the amount of the additional injury must determine the character of the result. The conclusive data in several experiments as well as the general mass of evidence obtained from all of them prove without question that within moderate degrees of injury a part regenerates more rapidly rather than less rapidly when it has regenerating company than when it regenerates by itself. The few exceptional cases showing a decrease in rate are capable of explanation on the grounds either of an incomplete control of subsidiary factors or of the presence of too high a degree of injury. The strength of the statement is made still stronger if possible by the consideration that additional injury to an individual involves a whole train of consequences likely to lead to disturb-

ance of vitality, to infection, and to other means of lessening the rate of regeneration. The accidents of the experiments in other words are all in the direction of an apparent lesser rate for the higher injury. These facts have already been sufficiently discussed under the heading of sources of error (pp. 514-518).

The above considerations seem to be a sufficient answer to the recent criticisms by Emmel, Scott, and Stockard. Stockard states (1) that the individual variation is sufficient to cause an overlapping of the rates in the two compared groups. (2) that in the case of animals with the molting habit this habit constitutes a serious source of error. (3) That even neglecting these difficulties the influence of degree of injury is small in amount as compared with such evident factors as the level of the cut.

The difficulties connected with the molting habit have already been mentioned on pp. 534-535, above and a paper dealing with several of the molting factors is in preparation. The other criticisms have been considered in discussing the sources of error. In a general way these criticisms are directed against the view that increase in injury brings about a decided increase in rate of regeneration. No special arguments against them are needed to support the statement that our evidence at present favors the view that increase in degree of injury to the individual within moderate degrees increases rather than decreases the rate of regeneration of a part.

The comparison that has been made between the effect of level of the cut in an organ and the degree of injury to the individual needs further attention. As stated above (p. 515), the level of the cut is not of much value for the determination of the effect of degree of injury to the individual because it involves changes in local conditions. Nor can the increase in rate of regeneration with a lowering of level be compared with the other increase. If the rate of regeneration of a chela for instance is unchanged by the simultaneous removal of the other one there is still twice as much regeneration being accomplished by the individual after a double removal as after a single one, just as in the case of a cut at a deeper level of the tadpole's tail proportionately more new tail is formed than at a higher level. The increase in rate dis-

cussed in the present paper involves more than a proportional increase.

The following general conclusion results naturally from the data as determined at present. The removal of a part of an animal involving a slight or moderate degree of injury is followed by a rate of regeneration that is less than it is in case the removal is accompanied by an additional removal of slight or moderate amount in other parts of the animal. If the degree of injury in either case is considerable, the additional injury results in a decrease in rate of regeneration of the part. For every part capable of regeneration, the statement can therefore be made that its rate of regeneration increases with increase in additional injury up to an optimum degree beyond which further injury causes a decrease in rate.

In a purely descriptive way it may be said that the increase in rate reveals the presence of a kind of inertia in the body of the animal comparable to ordinary physical inertia. The time necessary for the repair of an injury is not least in the case of the lowest injury but a certain degree of injury is necessary before the regenerative powers of the organism can work at their best. In the same way also in the case of the functional operculum of a Serpulid worm a considerable degree of injury is necessary to start the process of reversal of the opercula. The decrease in rate that follows at higher degrees of injury is undoubtedly due to another factor, the active disturbance of the ordinary mechanism of the body.

#### SUMMARY

- I An attempt was made to determine the pure effect of the degree of injury to the individual upon the rate of regeneration of a removed part.
- 2 The principal method consisted of the comparison of the rates of regeneration of an organ removed at a constant level when other parts of the animal were or were not removed at the same time.
  - 3 A special study was made of the various other factors influ-

encing the rate of regeneration in order that a more careful determination might be made of the effect of the degree of injury.

- 4 The data are divided into two groups including on the one hand the animals without a molting habit and on the other those with a molting habit. This was found advisable because the factor involved in molting cannot at present be fully controlled in most cases.
- 5 A summary is made of former experiments in the first group. These were made on the opercula of the Serpulid worms, Hydroides dianthus and Apomatus ampullifera, the arms of the brittle-star, Ophioglypha lacertosa and the oral arms of the Scyphomedusan, Cassiopea xamachana. They show that a removed organ regenerates less rapidly when it alone is removed than it does when other parts are removed at the same time.
- 6 The new experiments in the first group include five on the larvæ of the salamander, Amblystoma opacum and one on the tadpoles of the green frog, Rana clamitans. The organs whose rate of regeneration was studied are the tail and the fore- and hind-legs. The data confirm the former results by showing that additional injury to an individual is favorable to the regeneration of these organs.
- 7 In the group with a molting habit, a summary of fo mer experiments on the chelæ of Gelasimus pugilator and Alpheus dentipes and on the chelæ and walking legs of the adult Cambarus propinquus shows that the regeneration of a chela is less rapid when it alone is removed than it is when the other chela or the other chela and four walking legs are removed at the same time.
- 8 The new experiments yield data on the chelæ of the shrimp, Palæmonetes vulgaris; of two species of crabs, Porcellana platycheles and one of undetermined species, and of young individuals of the crayfish, Cambarus bartoni and Cambarus propinquus. They confirm the results of the former experiments.
- 9 The experiments as a whole show further that when the additional injury is excessive there is a decrease in the rate of regeneration of the organ under consideration.
- 10 The following general statement regarding the effect of the degree of injury to the individual upon the rate of regenera-

tion of an organ may be made: The rate of regeneration of an organ increases with increase in additional injury to the individual up to an optimum degree beyond which further injury leads to a decrease in rate. The position of the optimum is different in the different organs of an individual and in corresponding organs of different species. The amount of the optimum degree of additional injury is also changed with change in the level of removal in the organ under observation.

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#### APPENDIX

After the manuscript of this paper was sent to press the author received an important paper by Stockard¹ dealing in part with the question of the relation of the degree of injury to the rate of regeneration. Stockard has performed experiments on the arms of the Scyphomedusan, Cassiopea xamachana and the arms of the brittle-stars Ophiocoma riisei and Ophiocoma echinata. He concludes that "the medusa, Cassiopea, regenerates each oral arm at a rate which is independent of the degree of injury when replacing either one, two, four or six of its arms. If, however, eight arms are amputated, each arm is regenerated at a rate which, after taking account of the probable error, is significantly greater than the regeneration rates in medusæ injured to any less extent.

"The brittle star, Ophiocoma riisei, regenerates either one, two, three, four or all five arms at rates which are not significantly different. In other words, there is no relation between the rate of the individual arms and the degree of injury in this species.

"The rate of regeneration for individual arms in Ophiocoma echinata, another species of Ophiuran, is fastest when only a single arm is regenerating and successively slower when two, three, four and five arms are being replaced. The rate of regeneration is slower the greater the extent of injury."

In the first case, that of Cassiopea, Stockard finds that the animals decrease in size during the course of the experiments, the ones with a greater number of removed arms decreasing faster than those with a smaller number. He used the original disk diameters for the determination of the specific rates of regeneration. If the final disk diameters are taken the specific rate of regeneration of each arm on the whole increases with increase in number of removed arms. The question as to the use of the original or the final disk diameters depends upon the interpretation of

<sup>&</sup>lt;sup>1</sup> Studies on Tissue Growth. II. Journal of Experimental Zoölogy, vol, vi, pp. 433-469.

the cause of the decrease in size. If it is due to factors only secondarily connected with the removal, such as ability to get food, freedom in movement, etc., it seems to me that it is more valid to use the final rather than the original diameter as a basis. The decrease in size of the animals of necessity means a decrease in the rate of regeneration quite apart from the matter of degree of injury. The new arms are being regenerated in a proportion that is proper for the decreased disk and not for the original one. The smaller animal is certainly not regenerating arms of the original size. If the animals were fed an amount just sufficient to retain their original disk diameters it is probable that the specific regeneration would be greater in those with the greater number of removed arms.

The greatest degree of injury, however, in Stockard's experiments shows the greatest rate for the individual arms under either interpretation. In my Cassiopea materials the optimum came at a lower degree. The difference is evidently due to some factor in the conduct of the experiments, probably better living conditions in Stockard's animals.

In the brittle-star Ophiocoma riisei the same criticism may be offered of the use of the original rather than the final disk diameter though here it makes only a slight difference in the interpretation of the results. The data show very little if any difference in rate with a different number of removed arms.

In the brittle-star Ophiocoma echinata the data furnished by Stockard evidently show a decrease in rate of the individual arms with increase in the removed number. No criticism of the interpretation of the data occurs to the writer.

In comparing these brittle-star experiments with mine on Ophioglypha lacertosa it should be borne in mind that apart from specific differences there were also differences in the living conditions in the two cases. The level of the cut in the arms was higher up in Stockard's animals, the latter also had a considerable food supply and a much greater chance of active movement.

Stockard's three experiments thus give one case with increase, a second with no change and a third with a decrease in rate of regeneration as a result of increase in degree of injury. All three such instances are given in the data of my paper though the majority belong to the first group. His data therefore do not modify the general conclusion that on the whole the optimum degree of injury in an individual is not necessarily the lowest degree. In the majority of cases studied so far the optimum degree of injury is above the lowest degree.

The problem now is therefore not whether all animals under all conditions regenerate parts more rapidly or less rapidly with increase in number of removed parts. It seems to me to be the determination of the conditions affecting the location of the optimum and the relation of these conditions to the general problem of growth.

# SOME EXPERIMENTS ON THE EFFECT OF AGE UPON THE RATE OF REGENERATION<sup>1</sup>

BY

#### CHARLES ZELENY

#### INTRODUCTION AND GENERAL METHOD

The experiments described in the present paper were undertaken with the object of determining the relation between the age of an animal and its rate of regeneration. The general fact has been known since the time of Spallanzani that as a rule the regeneration of lost parts is not as rapid in old animals as in young ones.<sup>2</sup> It is not, however, always clear whether by this is meant the total time necessary for the replacement of a removed part or the actual rate of proliferation of the new tissues. Furthermore, exceptions to the general rule of decrease with age have been noted.<sup>3</sup>

The data to be given were obtained as a part of the study of several of the factors controlling the rate of regeneration. They are imperfect in that there is no instance of the working out of the rate during a complete life history. A full discussion of the general methods employed and the elimination of sources of error is given in the introductions to the papers on successive regeneration and the effect of the degree of injury in this number of the Journal of Experimental Zoölogy. The statements made there are applicable to the present experiments and need not be repeated here. The individual catalogue numbers are given in

<sup>&</sup>lt;sup>1</sup> Contributions from the Zoölogical Laboratory of Indiana University. No. 110.

<sup>2&</sup>quot;Die Regenerationsfähigkeit nimmt mit zunehmendem Alter eines Tierexemplares ab." Praibram, 1909. Experimental-Zoölogie 2. Regeneration, p. 221.

<sup>&</sup>lt;sup>3</sup> "Die scheinbare Unterbrechung auf mittleren Entwicklungsstadien in gewissen speziellen Fallen läszt sich auf den nachweislichen Wechsel in der Konsistenz oder der Gewebsverteilung zurückführen." Przibram, 1909, l. c., p. 221.

<sup>&</sup>lt;sup>4</sup> Successive Regenerations: additional observations and general discussion. This Journal. The effect of the degree of injury upon the rate of regeneration. This Journal.

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many of the tables of data in order to make possible the identification of animals used for more than one purpose.

In about one-half of the experiments the animals used were reared in the laboratory and the age is determined. In the cases in which the animals were not reared it is necessary to take the size of the animal as an indication of its age. The possibility of error in this connection is evident since individuals of the same size may be different in age.

As in the other papers on the factors controlling the rate of regeneration the data are divided into two groups including (A) those on non-molting and (B) those on molting animals. The molting habit introduces a factor which is difficult to control. The animals with this habit however possess some very obvious advantages. For a discussion of these points see the other papers in this number of the Journal.

#### EXPERIMENTS ON ANIMALS WITHOUT A MOLTING HABIT

# I The Regeneration of the Oral Arms in the Scyphomedusan, Cassiopea xamachanas

For the conditions of the experiment the reader is referred to the paper with the original data. The data bearing on the effect of age are given here in Table 1. The size of the animal is taken as an indication of its age.

In each of the six degrees of injury the average length of regenerated material is greater in the two larger individuals than in the two smaller ones. On the other hand the regenerated length per unit of disk diameter is less in the larger than in the smaller individuals. In other words when one or more oral arms are removed the actual proliferation of material is faster in the larger than in the smaller animals, but the increased rate is not sufficient to complete the removed arms as soon in the former as in the latter.

<sup>&</sup>lt;sup>5</sup> The full data are published in the paper on "The effect of degree of injury, successive injury and functional activity upon regeneration in the Scyphomedusan, Cassiopea xamachana." Journ. Exp. Zoöl., vol. v, no. 2. 1907.

## 2 The Arms of the Brittle-star, Ophioglypha lacertosa

The conditions of the experiment are given in the paper with the original data. The data bearing on the effect of age are given here in Tables 2, 3, 4 and 5. The disk diameter is taken as a measure of age with the reservation mentioned on the preceding page.

An examination of the tables shows that in general the actual regenerated arm lengths at any time are the greatest in individuals of medium size. Both the smaller and the larger individuals show shorter regenerated arms. When the regenerated length per unit of disk diameter is taken there is less difference between the smaller and medium individuals and more between the medium and larger ones. In other words the actual rate of regeneration in length is evidently greatest in medium sized individuals with a disk diameter of 12.5 to 15.0 mm. Both larger and smaller individuals regenerate new material less rapidly. The same is true (though not so markedly in the smaller individuals) for the specific length of regeneration. The smaller individuals ranging in size from 4.8 to 12.5 mm. therefore seem to constitute an exception to the general rule that the rate of regeneration decreases with age. This is true both of actual length and of specific length of regeneration. The data are not wholly satisfactory because of the large degree of variation and the small number of individuals. The larger individuals very evidently follow the rule of decrease both in actual length and in specific length of regeneration.

### 3 The Tail in Amblystoma jeffersonianum'

The data are taken from 181 individuals reared in the laboratory. A comparison is to be made especially between the younger individuals (Tables 6, 7 and 8) and the older individuals (Tables 9, 10 and 11). All the salamanders are still in the larval stage. The animals were used for the determination of other factors

The full data are published in the paper on "Compensatory Regulation," Journ. Exp. Zoöl., vol. ii, no. 1, 1905.

<sup>7</sup> Based wholly on new data.

than that of age and they therefore unfortunately are subject to the error of difference in length of the regeneration period. The rate of regeneration in length following an operation is at first slow, then increases rapidly to a maximum, after which it declines slowly to zero, which it reaches at the completion of regeneration. Table 6 is especially subject to this error because it covers a much longer period than the others. This factor however in no way invalidates the general conclusion to be drawn from the data.

The specific rate of regeneration. The average specific rate of regeneration of the tail in length is 0.0439 for individuals with a total body length of 22.6 mm., age of 28 days at the time of the operation and a regeneration period of 24 days; 0.0396 for those 26.5 mm. long, 34 days old and with a regeneration period of 15 days; and 0.0368 for those 26.8 mm. long, 48 days old and with a regeneration period of 15 days.

This is considerably greater than the specific rate for the older salamanders. For those 51.6 mm. long, 131 days old and with a regeneration period of 14 days the specific rate is 0.0148; for those 54.1 mm. long, 134 days old and with a regeneration period of 11 days it is 0.0126; and for those 51.6 mm. long, 135 days old and with a regeneration period of 10 days it is 0.0167.

The above applies to the cases in which nearly the whole tail was removed. A similar result is obtained when one-half of the tail is removed. The respective specific rates are then 0.048 0.042 and 0.038 for the younger and 0.020, 0.018 (-), and 0.014 (-) for the older animals.

Actual length of regenerated material. Not only is the specific rate less in the older than in the younger individuals but also the actual length of regenerated material is less.

The individuals with the whole tail removed are considered first. For age 28 days, length 22.6 mm. and regeneration period 24 days, the length of the regenerated tails is 9.36 mm. or 0.39 mm. per day; for age 34 days, body length 26.5 mm. and regeneration period of 15 days, the length of regenerated tails is 6.22 mm. or 0.41 mm. per day; and for age 48 days, length 26.8 mm. and regeneration period of 15 days the length of regenerated tails is 5.87 mm. or 0.39 mm. per day.

In older individuals the length of the regenerated tails is less; for age 131 days, length 51.6 mm. and regeneration period of 14 days it is 4.22 mm. or 0.30 mm. per day; for age 134 days, length 54.1 mm. and regeneration period of 11 days it is 2.96 mm. or 0.27 mm. per day; and for age 135 days, length 51.6 mm. and regeneration period of 10 days it is 3.4 mm. or 0.34 mm. per day.

A similar result is obtained when only one-half of the tail is removed. For age 28 days, length 22.6 mm. and regeneration period of 24 days in the one individual available the length of the regenerated tail is 7.7 mm. or 0.32 mm. per day; for age 34 days, length 27.3 mm. and regeneration period of 15 days in the six individuals available the average length of the regenerated tail is 3.8 mm. or 0.25 per day; and for age 48 days, length 26.7 mm. and regeneration period of 15 days it is 3.8 mm. or 0.25 mm. per day in the fourteen available individuals.

For the older individuals there is a decrease in the actual length and rate of regeneration as compared with the younger ones. For age 131 days, length 49.1 mm. and regeneration period of 14 days the average length of the regenerated tail in the four available individuals is 3.0 mm. or 0.21 mm. per day; for age 134 days, length 52.4 mm. and regeneration period of 11 days it is 2.3 (-) mm. or 0.21 (-) mm. per day; and for age 135 days, length 51.9 mm. and regeneration period of 10 days it is 1.75 (-) mm. or 0.17 (-) mm. per day.

The data as a whole show that in the salamanders 131 to 135 days old the rate of proliferation of new material in length is less than it is in salamanders 28 to 48 days old. The specific rate is obviously still more strikingly less than the actual rate of proliferation.

#### EXPERIMENTS ON ANIMALS WITH A MOLTING HABIT

## 4 The Chelæ of the Gulf-weed Crab, Portunus sayi:8

The age factor is discussed in detail in the original paper in which Tables 10, 11A and 12 refer especially to it. The general result only need be mentioned here. In 66 crabs ranging in cephalo-thoracic length from 3.9 to 14.5 mms. not only the rate of formation of new material but also the specific rate or rate per unit of cephalo-thoracic length increases with increase in size of the crab. The increase in specific rate is however associated with a corresponding increase in length of the normal uninjured chelæ in larger individuals. Perfectly normal chelæ should therefore be obtained as quickly in large (old?) individuals as in small (young?) ones between the sizes mentioned.

# 5 The Chelæ of Cambarus propinquus. (Tables 12 to 17)º

The data are taken from two series of young crayfish reared in the laboratory, one with 70 and the other with 93 individuals and one series of old crayfish with 74 individuals.

Two comparisons are made. The young individuals (Tables 12 and 13) are compared with adults (Tables 14 to 17) and among the adults the smaller individuals are compared with the larger ones (Tables 14, 15, 16 and 17).

Taking first the comparison between young animals and adults it is found that crayfish operated upon two days after the fourth molt have regenerated on the average 2.12 mm. of the propodite of the removed chela<sup>10</sup> in 14.6 days in two molts. The specific amount is 0.326 and the specific rate 0.0229. Those operated upon one day after the fourth molt have regenerated 1.86 mm.

The full data are published in the paper on "Regeneration in the Gulf-Weed Crab, Portunus sayi." Carnegie Institution Publications. Tortugas Laboratory Reports, vol. 2.

Based partly on new data and partly on data from the paper on "The effect of the degree of injury upon the rate of regeneration." Journ. Exp. Zoöl., vol. ii, 1905.

<sup>10</sup> Throughout the paper in speaking of the "length of the chela" the greatest length of the propodite is meant.

of chela in 13.8 days in two molts. The specific amount is 0.27 mm. and the specific rate 0.0195.

Adult crayfish on the other hand take a much longer period to complete a single molt and the propodite of the regenerated chela is on the average 6.47 mm. long in males and 6.14 mm. in females. This gives a specific amount of regeneration of 0.444 and a specific rate of 0.0049 for the males and a specific amount of 0.400 and a specific rate of 0.0030 for the females. Likewise in case both chelæ are removed the length of the regenerated chelæ is 6.4 for males and 6.07 for females and the corresponding specific amounts and specific rates are 0.435 and 0.0080 for the males and 0.404 and 0.0078 for the females.

The specific rates are therefore decidedly greater in the young crayfish than in the adults. The young however have had two molts as opposed to one in the adults.

The second comparison concerns the adults alone. If the smaller (younger?) adults are compared with the larger (older?) ones the actual regeneration lengths are greater in the larger than in the smaller ones. The specific amounts are practically alike in both groups. The specific rates however are slightly greater in the younger than in the older individuals, so that the former should complete their chelæ slightly sooner than the latter.

The data on Cambarus propinquus are unsatisfactory as regards the comparison between very young animals and adults because the regeneration periods under observation are so different. This criticism does not apply to the comparison between small and large adults.

When very young individuals are compared with adults there is evidently a greater rate of regeneration in the younger than in the older individuals not only in the sense of the time necessary to complete a new organ but also in the sense of rate of proliferation of new material. When younger and older adults are compared however, it is seen that the actual rate of proliferation is greater in the older individuals but the time necessary to complete the missing chela is nevertheless greater.

# 6 The Chelæ of Cambarus bartoni. (Tables 18 to 21)11

The crayfish used in these comparisons were all reared in the laboratory from one mass of eggs. Fifty individuals were obtained in this way.

Two comparisons are made, one between individuals with a single chela removed and the other between individuals in which both chelæ were removed. The comparison in each case is made between individuals operated upon one day after the fourth molt and others operated upon after the eighth, ninth or tenth molt. In each case the actual length of proliferated material is greater in the older individuals but the specific rate is less in the older than in the younger ones. The specific amounts of regeneration are nearly the same in the crayfish of all ages with a single chela removed and greater in the younger than in the older ones in the individuals with both chelæ removed.

The data as a whole favor the view that the actual rate of proliferation is greater in the older than in the younger individuals but not great enough to enable them to complete the lost part as soon as in the younger ones.

# 7 Palæmon tenuicornis. (Table 22)12

The data are obtained from a set of twenty-one individuals operated upon in June, 1906, at Tortugas. A comparison is made of the rate of regeneration of a single removed chela during one molt. Six individuals were taken during their third regeneration of a chela and three during their second regeneration. In each case the length of the regenerated chela increases with increase in size (age?) of the animal. The specific rate, however, decreases. For the specific amount of regeneration during the single molt no definite conclusion can be reached.

The data here as in the case of Cambarus bartoni show an increase in the rate of proliferation of material between the sizes of 6.8 and 13.1 mm. but this increase is not sufficient to enable

<sup>&</sup>quot; Based wholly on new data.

Based wholly on unpublished data.

the older ones to complete the whole organ as soon as it is completed by the younger ones.

# 8 Palæmonetes vulgaris. Nos. 1775–1805 and 1806–1861. (Tables 23, 24, 25 and 26)18

The individuals in each table are arranged according to cephalothoracic length. They range in size from 6.1 mm. to 13.6 mm.

In individuals Nos. 1755–1805 (Table 23) there is on the whole a slight increase in length of the regenerated chela during a molting period with increase in size, but this is probably associated with the increase in length of the molting period. In specific amount there is no evident difference in individuals of different size.

In individuals Nos. 1806–1861 (Tables 24 to 26), where the comparisons are made in every case eight days after the operation, there is on the whole no evident change in rate with change in size but the specific amount and rate of regeneration evidently decrease with age.

As far as any general conclusion may be drawn from the data on Palæmonetes vulgaris it may be said that there is no change in rate of regeneration of a removed organ with increase in size in the sense of rate of formation of new material. In specific rate there is either no change or a decrease in rate.

#### GENERAL RESULT

The data cover eight species of animals, Cassiopea xamachana, Ophioglypha lacertosa, Amblystoma jeffersonianum, Portunus sayi, Cambarus propinquus, Cambarus bartoni, Palæmon tenuicornis and Palæmonetes vulgaris.

In most cases the rate of regeneration of an organ in the sense of actual increase in length of the proliferating part is greater in older individuals than in younger ones. The comparison is however made only in individuals in which increase in size is an accompaniment of increase in age. Examples are: Cassiopea, Ophio-

<sup>48</sup> Based wholly on unpublished data.

glypha (up to 15 mm.), Portunus, Cambarus propinquus (10.6 to 19.0 mm.), Cambarus bartoni and Palæmon. In one case there is no evident change with age (Palæmonetes) and in a few there is a decrease with age (Ophioglypha above 15 mm., Amblystoma and Cambarus propinquus young as compared with adults).

The specific rate of regeneration or the rate per unit of length of the individual is however usually less in older than in younger individuals, so that it takes them longer to complete a removed appendage than in the younger ones. Examples are: Cassiopea, Ophioglypha, (above 15 mm.), Amblystoma, C. bartoni, C. propinquus, Palæmon, Palæmonetes (in part). In a few cases there is no change in specific rate with age (Ophioglypha, medium individuals, Portunus (?), Palæmonetes, in part), or there is an increase with age (Ophioglypha, smaller individuals, Portunus (?)16).

### DISCUSSION

The experiments as a whole confirm the general conclusion that in older individuals it takes longer to complete a removed organ than in younger ones.<sup>17</sup> This general result is usually evident in extremes of age without the use of any accurate measurements. As has been previously pointed out there are however undoubted exceptions to the rule, the most marked in the present set of experiments being the chelæ of Portunus and probably the younger individuals of Ophioglypha.

The accurate measurements of regenerating appendages has brought out the point upon which special emphasis needs to be laid, and this is the fact that in a majority of the species studied the actual rate at which new tissue is proliferated is greater in the older individuals than in the younger ones. The older individuals when they lose an appendage obviously have more material removed than do the younger ones, but the proportion of removed material to the whole mass of the body is not materially changed in those forms in which growth does not involve a change

<sup>14</sup> See special condition affecting the size of the chelæ, p. 568 of this paper.

<sup>15</sup> Data not conclusive.

<sup>&</sup>lt;sup>28</sup> See special conditions affecting the size of the chelæ, p. 568 of this paper.

<sup>17</sup> A full bibliography is given in Przibram. Experimental-Zoölogie. II. Regeneration. 1909.

in shape. In general the rate of regeneration in any individual increases with increase in amount of material removed. This is clearly shown by a study of the effect of the degree of injury and by such organs as the tail of the frog tadpole, where the rate is directly proportional to the distance of the cut from the tip. It follows therefore that if a length of the appendage in the older individuals equal to its total length in the younger ones were removed there would undoubtedly be on the whole a less rapid rate of regeneration than in the younger whole appendage. It also follows that if it were possible to conceive two animals of the same species, age, sex etc., and with the same environment but markedly different in size, the larger appendage in the larger one would be completed at the same time as the smaller appendage in the smaller one. Otherwise the above experiments show an increase in regeneration potential with age.

The age factor in those animals in which increase in age is accompanied by increase in size does not bring about a decrease in rate of proliferation when an appendage is removed, in the sense of the actual material produced, but a decrease as compared with a hypothetical young animal of the same size as the older one. The probable rate in this hypothetical, enlarged younger animal is based upon the data obtained from experiments on the rate of regeneration with different degrees of injury to the individual and from different levels of an appendage.

#### SUMMARY

- I In larger individuals of Cassiopea xamachana with a disk diameter of 26 to 42 mm. the actual proliferation of material in a regenerating arm is faster than in smaller individuals with a disk diameter of 8.4 to 21.0 mm. This greater rate, however, is not sufficient to complete the arms as soon in the older as in the younger individuals.
- 2 In Ophioglypha lacertosa individuals with a disk diameter of 12.5 to 15.0 mm. have the greatest rate of regeneration. Both smaller and larger individuals have a lower rate. The same is true of the rate per unit of disk diameter, though the difference

between the smaller and medium sized is not very pronounced and may not be significant.

- 3 In Amblystoma jeffersonianum, larvæ 131 to 135 days old as compared with those 28 to 48 days old regenerate the new tail less rapidly both when the whole and when half the tail is removed. This is true of the actual rate of increase in length as well as the rate per unit of total body length.
- 4 In individuals of Portunus sayi ranging from 3.9 to 14.5 mm. with increase in size of the animals there is an increase not only in the rate of proliferation but also in the specific rate. The increase in specific rate corresponds with an increase in specific size of the chelæ in uninjured individuals in such a way that in all crabs of the sizes studied new chelæ are completed in the same period of time.
- 5 Adult individuals of Cambarus propinquus regenerate less rapidly than young ones of the fourth to the sixth molt in actual rate of prolification as well as in time necessary to complete the removed organ. Among adult individuals ranging in length from 10.6 to 19.0 mm. the larger (older?) ones have a greater rate of regeneration but the time necessary to complete the removed part is on the whole greater in the older than in the younger ones.
- 6 In Cambarus bartoni all individuals were reared in the laboratory and their actual age was determined. The rate of regeneration is greater in the older individuals than in the younger ones but the specific rate is less and therefore the time necessary to complete the whole removed part is greater.
- 7 In Palæmon tenuicornis with cephalo-thoracic lengths varying from 6.8 to 13.1 mm. the same rule holds as for Cambarus bartoni.
- 8 In Palæmonetes vulgaris with cephalo-thoracic lengths ranging from 7.2 to 13.6 mm. there is no evident change in rate of proliferation of new material with change in size. The specific rate either shows no change or a decrease.
- 9 The general result is clear that in a majority of the cases the actual rate of proliferation of new tissue is greater in older than in younger individuals, but this is not sufficient to reproduce the removed organ as soon in the older as in the younger individuals.

TABLE 1

Cassiopea xamachana—Regeneration period, 24 days

	ьмат	LER INDIVI	DUALS	LAR	GER INDIVID	UALS
į	Size 8.4 to	21.0 mm. Av	.= 16.5 mm.	Size 26.0 to	42 mm. Av	= 39.9 mm.
Degree of injury	Disk diameter	Length of regener- ated arms	Specific amount of regenera- tion	Disk : diameter	Average length of regener- ated arms	of regen-
One arm at base	10.5	0.5	0.048	29 O 42.0	1.0	0.034
Average	13.7	0.75	0 053	35.5	2 0	0.052
Two arms at base	8.4	1.5	0.179 119	29 0 42 0	1.75 5.0	0.060
Average	14.7	2.0	0.149	35.5	3.37	0.089
Four arms at base .	11.2	2.0 3.0	0.179 0 194	35 ° 36 °	4·7 4 •	0.134
Average	13.3	2.5	0.186	35 5	4.35	0.122
Six arms at base	9 0 16.5	2.5 2.5	0.278	26 o 37.0	2.85 4·5	0.110
Average	12.7	2.5	0 214	31.5	3.67	0.116
Eight arms at base	10.8	2.0	0.185	28.0 34·5	2.0 3.0	0.072
Average	14.1	2.25	0.164	31.2	2.5	0.079
Whole mouth, apparatus including the eight arms	10.8	1.6	0.148	28.0 32.5	2.15	0.077
Average	13.6	1.8	0.134	30.2	3 · 57	0.115

Table 1. Under each degree of injury there were five individuals in the experiment. The two smallest are compared with the two largest, the medium individuals not being included in the present table. The animals were not fed during the experiment. The specific amount of regeneration is the regenerated arm length per unit of disk diameter.

TABLE 2

Ophioglypha lacertosa—One arm removed at base

	22 1	DAYS	33	DAYS	46		
Disk diameter	Length of regenerat- ing arm	Specific amount of regenera- tion	Length of regenerat- ing arm	Specific amount of regenera- tion	Length of regenerat- ing arm	Specific amount of regenera- tion	Remarks
4.8	0.15	0.03	0.2	0.04	0.0		
6.5	0.45	0.07	0.1	0.01	0.0		
6.6	1.4	0.21	2.4	0.36	3.0	0.45	
11.0	0.6	0.05	2.0	0.18	2.0	0.18	
11.2	2.0	0.18	3.0	0.27	4.0	0.36	
13.2	0.2	10.0	2.0	0.15	3.1	0.23	
13.5							dead (22)
14.5	1.0	0.07	1				dead (33)
19.8	0.0		0.0		0.0	1	

Tables 2, 3, 4 and 5. The animals are arranged in order of size. The specific amount of regeneration is the regenerated length per unit of disk diameter. The individuals were not fed during the course of the experiment.

TABLE 3

Ophioglypha lacertosa—Two arms removed at base

	22 DAY8		33 DAYS		46		
Disk diameter	Average length of regenerat- ating arms	Specific amount of regenera- tion	Average length of regenerat- ing arms	Specific amount of regenera- tion	Average length of regenerat- ing arms	Specific amount of regenera- tion	Remarks
6.0							dead (22
6.5	0.55	0.08	0.45	0.07	0.0		•
8.7	1.9	0.22	1.9	0.22	2.0	0.23	
10.8	1.8	0.17	1.6	0.15	0.65	0.06	
13.0					_	į !	dead (22)
13.5	1.25	0.09	2.3	0.17	3.5	0.26	
14.0	1.8	0.13	3.0	0.21	3.3	0.24	
15.0	0.85	0.06	2.6	0.17	4.05	0.27	
19.3	0.0		0.0		0.0		

TABLE 4

Ophioglypha lacertosa—Three arms removed at base

						~	
	22 1	DAYS	33 1	DAY5	<b>4</b> 6 p	AYS	
Disk diameter	Average length of regenerating arms	Specific amount of regenera- tion	Average length of regenerat- ing arms	Specific amount of regenera- tion	Average length of regenerat- ing arms	Specific amount of regenera- tion	Remarks
5.5				,			dead (22)
6.5	0.6	0 09	1.05	0.16	'		dead (46)
90						1	dead (22)
11.0	1.15	0 10	1 75	0.16	3.05	0.28	
12.5	1 35	0.11	2.35	0.19	3.2	0.26	1
12.5	2.25	o 18	4.25	0.34	6.65	0.53	
13 8	1.1	0 08	3 4	0.25	4.9	0.36	i
14.3	1.1	0.08	1.7	0 12	2.15	0.15	
20.0	0.05	0 00	0.35	0 02	10	, 0 05	ı

TABLE 5

Ophioglypha lacertosa — Four arms removed at base

	22 D	AYS	33 1	PAYS	46 DAYS		
Disk diameter	Average length of regenerating arms	Specific amount of regeneration	Average length of regenerating arms	Specific amount of regeneration	Average length of regenerating arms	Specific amount of regeneration	
4.9	.65	0.13	1.0	0.20	0.45	0.09	
6.0	1.0	0.17	1.65	0.27	1.9	0.32	
8.2	2.45	0.30	4.6	0.56	6 2	0.76	
11.3	1.85	0.16	3 .65	0.32	5.3	0.47	
12.3	2.15	0.17	4.3	0.35	7.1	0.58	
12.8	0.6	0.05	2.1	0.16	4.05	0.32	
12.8	1.55	0.12	2.9	0.23	4.85	0.38	
15.2	1.75	0.12	3.3	0.22	5.55	0.37	
18.3	0.1	0.05	1.75	0.10	3.65	0.20	

TABLE 6

Amblystoma jeffersonianum. Nos. 2032-2076. First regeneration. Younger individuals. Regeneration period = 24 days (February 17 to March 13, 1907). Age at time of operation = 28 days

CATA- LOGUE NUMBER	PART OF TAIL REMOVED (APPROX.)	TOTAL LENGTH OF ANIMAL	LENGTH OF REMOVED TAIL	LENGTH OF REGENERATED TAIL	SPECIFIC AMOUNT OF REGENERA- TION	SPECIFIC RATE OF REGENERA TION
2033	whole	22.0	8.4	9.5	1.13	0.047
2039	whole	20 0	8.1	8.1	1.00	0.042
2043	whole	24.0	9.8	. 8.1 ,	0.83	0.035
2046	whole	23.0	9.1	10.0	1.10	0.046
2049	whole	25.0	10.1	10.6	1.05	0.044
2053	whole	21.5	8.4	10.6	1.26	0.052
2058	whole	21.5	8.5	9.2	1.08	0.045
2061	whole	22.0	8.5	8.6	1.01	0.042
2072	whole	24.0	9.4	9.4	1.00	0.042
2076	whole	23.0	9.1	9.5	1.04	0.044
verage		22.6	8.94	9.36	1.05	0.0439
2054	half	19.0	6.7	7.7	1.15	0.048

Tables 6 to 11. The eggs were collected on the morning of January 20, 1907, and were probably laid during the previous night, all being in early cleavage stages at the time of collection. Nos. 2000 to 2014 are exceptions, being collected on January 8. The specific amount of regeneration is the amount per unit of removed tail. The final measurements made on June 20 are from the animals as killed in Gilson's fluid and preserved in 85 per cent alcohol. In Table 10 "no" regeneration means less than 2 mm. in, Table 11 it means less than 1 mm.

TABLE 7

Amblystoma jeffersonianum. Nos. 2172-2214. First regeneration. Younger individuals. Regeneration period = 15 days (February 23 to March 10, 1907). Age at time of operation = 34 days

CATA- LOGUE NUMBER	PART OF TAIL REMOVED (APPROX.)	TOTAL LENGTH OF ANIMAL	LENGTH OF REMOVED TAIL	LENGTH OF REGENER- ATED TAIL	SPECIFIC AMOUNT OF REGENERA- TION	SPECIFIC RATL OF REGENERA- TION
2172	whole	27.5	11.3	6.3	0.56	0.037
2174	whole	26.0	10.5	7.1	0.68	0.045
2180	whole	25.0	9.0	6 9	0.77	0 051
2182	whole	27.0	10.6	6.3	0.59	0.039
2191	whole	28.0	11.3	5.5	. 0.49	0.033
2201	whole	27.0	10.2	6.1	0.60	0.040
2204	whole	22.5	9.6	4.6	0.48	0.032
2213	whole	29.0	11.7	7.0	0.60	0.040
Average.		26.5	10.52	6.22	0.596	0.0396
2183	half	28.0	6.5	4.2	0.65	0.043
2186	half	26.0	5.6	4.2	0.75	0.050
2196	half	27.0	5.5	3-3	0.60	0.040
2199	half	28.5	6.8	3 9	0.57	0.038
2208	half '	28.0	6.5	3 - 3	0.51	0.034
2212	half	26.5	5.7	4.1	0.72	0.048
Average	.'	27.2	6.1	2.8	0.62	0.0422

TABLE 8

Amblystoma jeffersonianum. Nos. 2077-2171. First regeneration. Younger individuals. Regeneration period = 15 days (March 9 to March 24, 1907). Age at time of operation = 48 days

CATA-	PART OF	: }		LENGTH OF	SPECIFIC	SPECIFIC
LOGUE	TAIL REMOVED	TOTAL LENGTH	LENGTH OF	REGENER-	AMOUNT OF	RATE OF RE-
NUMBER	(APPROX.)	OF ANIMAL	REMOVED TAIL	ATED TAIL	REGENERA-	GENERA-
NUMBER	(ALL KOAL)		,		TION	TION
2078	whole	28.0	12.0	6.1	0.51	0.034
2080	whole	24.0	9.8	5.7	0.58	0.039
2085	whole	27.5	11.0	6. т	0.55	0.037
2088	whole	27.0	10.0	4.6	0.46	0.031
2091	whole	26.5	10.9	4.5	0.41	0.027
2101	whole	29.0	12.0	6.1	0.51	0.034
2103	whole	25.0	10.0	3.8	0.38	0.025
2111	whole	24.5	9.7	5.6	0.58	0.039
2118	whole	29.0	11.7	7.5	0.64	0.043
2121	whole	26.0	10.0	4.4	0.44	0.029
2123	whole	28.0	0.11	6.8	0.62	0.041
2128	whole	27.0	10.2	4.9	0.48	0.032
2132	whole	25.0	9.7	5.4	0.56	0.037
2134	whole	27.0	10.4	7.0	0.67	0.045
2141	whole	28.0	11.5	4.5	0.39	0.026
2152	whole	28.0	11.2	7 2	0 64	0.043
2154	whole	27.5	11.2	5.9	0.53	0.035
2158	whole	27.0	10.8	6.7	0.62	0.041
.2160	whole	26.0	10.0	7.0	0.70	0.047
2165	whole	25.5	10.0	6.2	0.62	0.041
2168	whole	27.0	10.2	7.2	0.71	0.047
verage		26.79	10.63	5.87	0.552	0.0368
2084	half	28.0	7.2	3.8	0.53	0.035
2087	half	25.5	6.5	3.8	0.58	0.039
2094	half	24.0	6.4	2.7	0.42	0.028
2098	half	27.5	7.1	3.7	0.52	0.035
2107	half	27.0	6.9	4.2	0.61	0.041
2112	half	26.0	6.5	3.6	0.55	0.037
2119	half	29.5	7.9	3.8	0.48	0.032
2124	half	27.5	5.6	3.0	0.54	0.036
2133	half	26.5	6.6	3.9	0.59	0.039
2135	half	25.0	6.0	3.9	0.65	0.043
2138	half	27.5	7.1	5.3	0.75	0.050
2150	half	27.5	6.1	4.0	0.66	0.044
2156	half	26.0	6.4	4.0	0.62	0.041
2170	half	27.0	6.0	3-5	0.58	0.039
Average		26.75	6.59	3.80	0.577	0.0385

TABLE 9

Amblystoma jeffersonianum. Nos. 2032-2076. First regeneration. Older individuals. Regeneration period = 14 days (June 6 to June 20, 1907). Age at time of operation = 131 days

CATALOGUE NUMBER	PART OF TAIL RE- MOVED (APPROX.)	TOTAL LENGTH OF	TAIL LENGTH	LENGTH OF REMOVED TAIL	LENGTH OF REGENER- ATED TAIL	SPECIFIC AMOUNT OF REGENERA- TED TAIL	SPECIFIC RATE OF REGENERA- TION
2032	whole	51.5	24.5	18.5	4.2	0.23	0.016
2042	whole	52.0	25.7	20.2	5.0	0.25	810.0
2055	whole	48.5	24.4	20.2	3.6	0.18	0.013
2056	whole	52.1	26.0	20.4	3.2	0.16	0.011
2074	whole	54.0	27.1	23.1	5.1	0.22	0.016
Average		51.62	25.54	20.48	4.22	0.208	0.0148
2048	half	48.3	22.5	11.5	2.8	0.24	0.017
2057	half	51.7	25.3	13.8	3.5	0.25	0.018
2064	half	47.5	23.5	10.4	3.0	0.29	0.021
2062	third	49.0	24.9	8.1	2.7	0.33	0.024
Average		49.12	24.05	10.95	3.0	0.277	0.020

TABLE 10

Amblystoma jeffersonianum. Nos. 2077-2171. First regeneration. Older individuals. Regeneration period = 11 days (June 9 to June 20, 1907). Age at time of operation = 134 days.

CATA- LOGUE NUMBER	PART OF TAIL REMOVED	TOTAL LENGTH OF ANIMAL	TAIL LENGTH	LENGTH OF REMOVED TAIL	LENGTH OF REGENER- ATED TAIL	SPECIFIC AMOUNT OF REGENER- ATED TAIL	SPECIFIC RATE OF REGENER- ATION
2081	whole	52.5	27.0	22.2	3.9	0.18	0.016
2090	whole	51.2	24.9	19.4	3.6	0.19	0.017
2099	whole	56.0	27.8	21.2	2.0	0 09	0.008
2105	whole	55.0	28.2	23.0	1.8(;)	0.08	0.007
2110	whole	53.3	25.9	20.0	2.5	0.12	0.011
2117	whole	59.1	29.6	24.0	4.0	0.17	0.015
2126	whole	55.0	27.9	22.0	3.3	0.15	0.014
2130	whole	52.1	25.4	20.5	2.0	0.10	0.009
2142	whole	50.8	24.3	18.9	2.8	0.15	0.014
2147	whole	56.0	27.8	23 I	3.7	0.16	0 015
verage		54.1	26.88	21.43	2.96	0.139	0.0126
2082	half	53 · 7	26 9	15.0	2.0	0.13	0.012
2100	half	54.6	26.9	12.2	none		
2108	half	41.2	19.9	8.8	2.5	0.28	0.025
2114	half	56.0	29 2	13.9	2.6	0.19	0.017
2122	half	58.3	29.3	12.9	none		
2136	half	50.0	25.6	12.9	none		
2151	half	51.6	25 3	11.1	2.1	,0.19	0.017
2164	half	54.2	26 8	12.8	none	,	
verage		52.45	26.24	12.45			0.0177(

TABLE 11

Amblystoma jeffersonianum. Nos. 2172-2214. First regeneration. Older individuals. Regeneration period = 10 days (June 10 to June 20, 1907). Age at time of operation = 135 days

CATALOGUE NUMBER	PART OF TAIL REMOVED (APPROX.)	TOTAL LENGTH OF ANIMALS	TAIL LFNGTH	LENGTH OF REMOVED TAIL	LENGTH OF REGENER- ATED TAIL	SPECIFIC AMOUNT OF REGEN- ERATION	SPECIFIC RATE OF REGENER- ATION
2175 2181 2185 2193 2198 2203 2207 2214	whole whole whole whole whole whole whole whole	50.4 53.8 54.1 48.4 53.2 53.6 50.5	24.2 26.1 27.9 23.5 25.9 27.6 25.4 24.1	20.0 20.4 21.1 18.5 21.0 21.3 21.2	3.7 4.4 3.0 2.8 1.5 4.0 3.8 4.3	0.18 0.22 0.14 0.15 0.07 0.19 0.18	o.018 o.022 o.014 o.015 o.007 o.019 o.018
			25.6	20.5	3.4	0.167	0.0167
2177 2188 2194 2205 2209	half half half half half	56.2 54.1 49.8 47.4 52.1	28.7 27.7 23.4 24.4 26.6	13.3 12.1 11.0 11.3 11.5	2.2 1.0(?) 2.2 none 1.6	0.16	0.016 0.008 0.020 0.014
Average		. 51.9	26.2	11.8		!	0.0145(-)

TABLE 12

Cambarus propinguus. Nos. 2300–2378. Right chela removed 2 days after 4th molt

CATALOGUE NUMBER	SEX	MOLTS	DAYS: OPERATION TO 6TH MOLT	CEPHALO-THORACIC LENGTH, 4TH MOLT. (APPROX.)	CEPHALO-THORACIC LENGTH, 6TH MOLT	LENGTH OF UNINJURED CHELA, 6TH MOLT	LENGTH OF REGENERATED RIGHT CHELA, 6TH MOLT	SPECIFIC AMOUNT OF REGENER ATION	SPECIFIC RATE OF REGENERA-	LENGTH OF REGENERATING CHELA PER UNIT OF UNIN- JURED CHELA	LENGTH REGENERATED FER DAY, PER UNIT OF UNIN-
2319	♂	4-6	22(-)	5.4	7.03	3.55	2.29	0.33	0.015	0.65	0.030
2325	♂	4-6	15	5.4	5-97	3.18	2.04	0.34	0.023	0.64	0.043
.2331	♂	4-6	12	5.4	6.47	3.40	1.78	0.28	0.023	0.52	0.043
2338	ď	4-6	13	5.4	6.51	3.60	2.13	0 33	0.025	0.59	0.045
2339	♂	4-6	15	5.4	6.23	3.19	2.22	0.35	0.023	0.70	0.047
2354	₽	4-6	14	5.4	6.55	3.18	2.07	0.32	0.023	0 65	0.046
2358	ę	4-6	12	5.4	6.59	3.03	2.03	0.31	0.026	0.67	0.056
2364	ď	4-6	14	5-4	6.81	3.55	2.38	0.35	0.025	0.67	0.048
erage			14.6	5-4	6.52	3 · 33	2.12	0.326	0.0229	0.636	0.045

Table 12. In all chela measurements in this and the following tables "chela length" means the greatest length of the propodite. The specific amount of regeneration is the amount per unit of cephalo-thoracic length. The average length of the right chela in a control series of 17 unoperated individuals was 3.41 mm. at the sixth molt.

TABLE 13

Cambarus propinquus. Nos. 2391-2483. Right chela removed one day after fourth molt

CATALOGUE NUMBER	9EX	MOLTS	DAYS: OPERATION TO 6TH MOLT	CEPHALO-THORACIC LENGTH, 4TH MOLT (APPROX.)	CEPHALO-THORACIC LENGTH, 6TH MOLT	LENGTH OF UNINJURED CHELA, 6TH MOLT	LENGTH OF REGENERATED RIGHT CHELA, 6TH MOLT	SPECIFIC AMOUNT OF REGENER-ATION	SPECIFIC RATE OF REGENER- ATION	LENGTH OF REGENERATED CHELA PER UNIT OF UNINJURED CHELA	LENGTH REGENERATED PER DAY PER UNIT OF UNIN-
2402	ę	4-6	14	5 3	6.72	3.65	1.67	0.25	0.018	0.46	0.033
2406	♂	4-6	12	5-3	6.69	3.34	1.82	0.27	0.022	0.54	0.045
2409	♂	4-6	13	5.3	6.88	3.50	1.64	0.24	0.018	0.47	0.036
2412	ਰੋ	4-6	13	5.3	7.22	3.90	1.70	0.24	810.0	0.44	0.031
2435	ੌ	4-6	16	5.3	7.14	3.72	2.13	0.30	0.019	0.57	0.036
2477	Ç	4-6	15	5 - 3	6.58	3 · 53	2.20	0.33	0.022	0.62	0.041
Average			13.8	5.3	6.87	3.61	1.86	0.27	0.0195	0.52	0.037

Table 13. The average length of the right chela in a control series of 18 unoperated individuals was 3.52 mm. at the sixth molt.

TABLE 14

Cambarus propinquus. Adult males. Right chela removed

CATALOGUE NUMBER	DAYS: OPERATION TO MOLT	CEFHALO-THORACIC LENGTH	LENGTH OF UNINJURED LEFT	LENGTH OF REGENERATED RIGHT CHELA	SPECIFIC AMOUNT OF REGENER-ATION	SPECIFIC RATE OF REGENERA-	LENGTH OF REGENERATED CHELA PER UNIT OF UNIN- JURED CHELA	LENGTH REGENERATED PER DAY PER UNIT OF UNIN- JURED CHEIA
737	71	10.9	6.7	4.7	0.431	0.0061	0.70	0.010
806	86	11.5	6.7	4.6	0.400	0.0047	0.69	0.008
797	57	12.2	7.6	5.7	0.467	0.0082	0.75	0.013
744	108	13.3	7.8	5.9	0.444	0.0041	0.76	0.007
745	58	13.7	8.6	6.3	0.460	0.0079	0.73	0.013
736	72	14.2	9.7	6.0	0.423	0.0059	0.62	0.009
804	92	14.9	9.9	6.9	0.463	0.0050	0.70	0.008
762	107	15.2	9.9	6.85	0.451	0.0042	0.69	0.006
778	105	15.5	8.9	6.7	0.432	0.0041	0.75	0.007
777	137	15.9	10.3	7.3	0.459	0.0034	0.71	0.005
735	137	16.0	12.4	7.0	0.437	0.0032	0.56	0.004
754	106	16.1	14.0	7.6	0.472	0.0045	0.54	0.005
761	116	16.6	11.8	7.0	0.422	0.0036	0.59	0.005
752	112	17.7	13.0	8.0	0.452	0.0040	0.62	0.006
verage.				6.47	0.444	0.0049	0.67	0.0076

Tables 14, 15, 16, and 17. Adult males and females were collected and operated upon without waiting for the appearance of a molt. This accounts for the great variation in the period from the operation to the first molt. The individuals are arranged in order of cephalo-thoracic length.

TABLE 15
Cambaius propinquus. Nos. 733-806. Adult females. Single chela removed.

CATA- LOGUE NUMBER	DAYS: OPERATION TO MOLT	CEPHALO- THORACIC LENGTH	LENGTH OF UNINJURED LEFT CHELA	LENGTH OF REGENERATED RIGHT CHELA	SPECIFIC AMOUNT OF REGENERA- TION	SPECIFIC RATE OF REGENERA- TION
791	135	11.0	6.4	4.6	0.418	0.0031
807	140	11.3	5.6	3.8	0.336	0.0024
775	119	14.0	9.1	6.0	0.429	0.0036
785	104	14.6	8.3	6.3	0.432	0.0042
743	106	15.0	9.1	6.4	0.427	0.0040
784	163	15.2	9.6	6.0	0.395	0.0024
738	108	15.4	9.0	6.9	0.448	0.0042
760	165	15.7	9.4	6.0	0.382	0.0023
799	142	15.9	10.4	6.8	0.427	0.0030
796	133	16.2	10.6	6.7	0.414	0.0031
751	. 153	17.0	11.1	6.2	0.365	0.0024
805	167	17.0	10.5	6.1	0.359	0.0021
770	181	18.0	10.0	7.0	0.389	0.0021
759	144	18.8	12.2	7.1	0.378	0.0026
verage		-	••••	., 6.14	0.400	0.0030

TABLE 16

Cambarus propinquus. Nos. 733-806. Adult males. Both chelæ and last two pairs of walking legs removed

CATA- LOGUE	DAYS:	CEPHALO- THORACIC	LFNGTH OF	REGENERAT	ING CHULE	* SPECIFIC AMOUNT OF	SPECIFIC RATE OF
NUMBER	TO MOLT	LENGTH	Right	Left	Average	REGENERA-	REGENERA- TION
789	34	11.0		4.6	4.6(?)	0.418	0.0123
739	33	12.2	5-4	5.2	5.3	0.434	0.0132
792	44	13.4	5.2	5.2	5.2	0.388	0.0088
803	42	13.5	5.6	5.0	5.3	0.393	0.0094
801	65	14.3	6.65	6.75	6.7	0.469	0.0072
740	76	14.7	6.8	6.7	6.75	0.459	0.0060
764	84	14.7	7.1	7.1	7.1	0.483	0.0057
780	44	14.8	7.2	6.8	7.0	0.473	0.0107
790	48	15.5	6.7	6.7	6.7	0.432	0.0090
748	83	15.8	· 6.4	6.3	6.35	0.402	0.0048
732	69	16.5	6.4	6.3	6.35	0.385	0.0056
773	95	16.9	7.6	7.6	7.6	0.450	0.0047
765	73	17.5	8.3	8.2	8.25	0.471	0.0065
verage					. 6.4	0.435	0.0080

TABLE 17

Cambarus propinquus. Nos. 733-806. Adult females. Both chelæ and last two pairs of walking legs removed

CATA- LOGUE	DAYS:	CEPHALO-	IFNGTH O	FREGENERA	TING CHELÆ	SPECIFIC AMOUNT OF	SPECIFIC RATE OF
NUMBER	TO MOIT	LENGIH	Right	Left	Average	RFGENERA- TION	REGENERA- TION
734	27	11.8	4 9	4 9	4 9	0 415	0.0154
741	29	119		4 7	4.7 (?)	0 395	0.0136
794	37	12 4	5.3	5 0	5 15	0.415	0 0112
742	31	12 6	5 5	5 4	5 45	0.433	0 0140
747	31	13.5	6 2	6 i	6 15	0.456	0.0147
795	35	13.5	5.6	5.4	5 5	0 407	0.0116
787	32	13.7	5 6	5.7	5 65	0 412	0.0129
800	37	13 9	6 ı	5 9	6 0	0 432	0.0117
793	33	140	5 3	5.4	5 35	0 382	0.0116
772	τ18	14.0	6.1	6 3	6 z	0.443	0.0038
779	108	15.0	56	5 6	56	0 373	0 0035
769	32	15 2	6.2	6 2	6 2	0.408	0 0127
731	134	16.1	6 8	6 9	6.85	0 425	0.0032
749	117	16 4	6 1	6.2	6.15	0.375	0.0032
763	52	16.9	6 7	6 9	6 8	0.402	0.0077
755	121	17.3	6 8	7 0	6 9	0.399	0 0033
771	115	17.8	7 2	7 0	7.1	0.399	0.0035
756	143	17.8	7.0	6 9	6 95	0 390	0.0027
782	148	18.8.	7 0	7 4	7.2	0 383	0.0026
750	147	19.0	6 8	6 3	6.55	0 345	0.0023
erage					6.07	0.404	0.0078

TABLE 18

Cambarus bartons. Nos. 2218-2267. Single chela removed. Regeneration during molts four to six

CATA- LOGUE	MOLTS	INTERVAL	AVERAGE CEPHAIO- THORACIC LENGTH MOLTS 4, 5	LENGTH OF UNINJURED CHELA	LENGTH OF REGENERAT- ING CHELA	SPECIFIC AMOUNT OF REGENERA- TION	SPECIFIC RATE OF REGENERA- TION
2223	4-5-6	23	6.5	4.0	3.5	0.54	0.023
2262	4-5-6	19	6.5	4.7	2.9	0.45	0.024
2218	4-5-6	23	6.6	4.7	2.9	0.44	0.019
2219	4-5-6	26	6.6	4.6	3.5	0.53	0.020
2259	4-5-6	19	6.6	4.9	3.0	0.45	0.024
2239	4-5-6	31	6.7	4.4	3 2	0.48	0.015
2248	4-5-6	24	6.7	4.4	3.1	0.46	0.015
2227	4-5-6	20	6.8	5.1	3.6	0.53	0.0265
2238	4-5-6	23	6.8	5.0	3.6	0.53	0.023
2252	4-5-6	28	6.8	4.8	3.5	0.51	0.018
L2242	4-5-6	20	6.9	5.5	3 1	0.45	0.0225
verage		23 3	6.68	4.74	3.26	0.488	0.021

Tables 18, 19, 20, and 21. The specific amount of regeneration is the length of the regenerated chela propodite per unit of cephalo-thoracic length.

TABLE 19

Cambarus bartons. Nos. 2218-2267. Single chela removed. Regeneration during molts eight to nine and nine to ten

CATA- LOGUE NUMBER	MOLTS	INTERVAL IN DAYS	CEPHALO- THORACIC LENGTH		LENGTH OF REGENERA- TING CHELA	SPECIFIC AMOUNT OF REGENERA- TION	SPECIFIC RATE OF REGENERA- TION
2241	8-9	27	10.5	7.0	5.3	0.50	0.019
2246	8-9	30	10.5	7.1	5.7	0.54	0.018
2267	8-9	20	10.5(?)	6.7	4.4	0.42	0.021
2221	9-10	27	11.5	7.4	5-4	0.46	0.017
2220	9-10	43	11.7	8.0	6.0	0.50	0.012
erage		. 29.4	10.94	7.24	5.36	0.484	0.017

TABLE 20

Cambarus bartons. Nos. 2218-2267. Both Chelæ removed. Regeneration during molts four to six

CATA-	-		CEPHALO-	I ENGTH OF	REGENER	ATING CHELÆ	SPECIFIC	SPECIFIO
LOGUE NUM- BER	MOLTS	INTERVAL IN DAYS	LENGTH MOLTS 4, 5	Left	Right		AMOUNT OF	RATE OF REGENERA- TION
2253	4-5-6	20	6.7	3.1	3.0	3.05	0.46	0.023
2245	4-5-6	26	6.9	4.0	4.0	4.0	0.58	0.022
2237	4-5-6	27	7.1	4.2	4.2	1 4.2	0.59	0.022
Average .		24.3	6.9	3.77	3 · 73	3 - 75	0.543	0.0223

TABLE 21

Cambarus bartons. Nos. 2218-2267. Both chelæ removed. Regeneration during molts 8-9, 9-10

and 10-11

CATA- LOGUE	MOLTS	INTERVAL	CEPHALO-	LENGTH OF	REGENERAT	ING CHELA	SPECIFIC AMOUNT OF	
NUM- BER		IN DAYS	LENGTH	Left	Right	Average	REGENERA- TION	REGENERA- TION
2228	8-9	22	10.2(?)	5.1	5.0	5.05	0.48	0.022
2240	8-9	20	10.5	4.I	4.5	4-3	0.41	0.0205
2222	8-9	27	10.9	5-4	5.5	5.45	0.50	0.019
2258	9-10	25	11.9	5.9	6.0	5.95	0.50	0.020
2233	10-11	30	12.1	6.8	6.6	6.7	0.55	0.018
Average .		24.8	11.12	5.46	5.52	5 · 49	0.488	0.020

TABLE 22
Palæmon tenuicornis. Nos. 1432-1452

CATA- LOGUE NUMBER	FINAL CEPHALO- THORACIC LENGTH	FINAL REGENERA- TION		LENGTH OF REGENER- ATED CHELA	LENGTH OF MOLTING PERIOD	SPECIFIC AMOUNT OF REGENERA- TION	BPECIFIC RATE OF REGENERA- TION
1432	6.8	third.	?	2.01	8	0.296	0.044
1441	7.4	third	2.95	2.52	10	0.341	0.046
1445	9.7	third	4.18	3.63	8	0.374	0.039
1449	10.6	third (?)	4.25	3.46	11	0.326	0.031
1450	11.0	third	3.81	3.12	8	0.284	0.026
1452	13.1	third	6.56	5.00	10	0.382	0.029
1442	7.6	second	2.80	2.56	10	0.338	0.044
1439	8.0	second	?	2.81	11	0.351	0.044
1451	8.11	second	5-03	4.20	12	0.356	0.030

Table 22. The specific amount of regeneration is the regenerated chela propodite length divided by the final cephalo-thoracic length, i. e., the length regenerated per unit of cephalo-thoracic length. The length of the uninjured chela is not as good a standard as the body length because its length may be affected by the removal.

TABLE 23

Palæmonetes vulgaris. Nos. 1755-1805. Regeneration of the chelæ in individuals 7.2 to 13.6 mm.
in cephalo-thoracic length

<b>2</b>	ING US I	104 1674	DENGTH OF ORIGI-		EASURE-	1	FINAL MEA	SUREMENT	
CATALOGUE	FINAL MOLTING PERIOD. MINUS	FINAL CEPHALO- THORACIC LENGTH	NAL RE- MOVED CHELA L= LEFT R=RIGHT	uninjured	Length of regenerated chela		- <del></del>	Second re	Sp. amt.
	A (			_		Dengin	ор. али.		op. ame.
1777	21	7.2	12.01	2.06	1.25	1.93	0.27	1.93	0.27
1771	12	8.9	r 2.77	2.83	2.19	1.45	0.16	1.67	0.19
1765	8	9.5	r 2.73	2.85	1.61	1.32	0.14	1.31	0.14
1779	12	9.8	12.69	2.98	2.38	1.93	0.20	1.89	0.19
1758	10	9.9	r 2.79	2.83	1.75	1.96	0.20	1.87	0.19
1778	12	9.9	12.83	3.14	1.93	1.63	0.16	1.67	0.17
1791	17	9.9	12.96	3.06	2.43	2.40	0.24	2.39	0.24
1761	11	10 0	r 2.87	3.12	1.99	2.07	0.21	2.02	0.20
1780	11	10.0	13.15	3.23	2.38	1 78	0.18	1.78	0.18
1789	18	10.0	13.18	3 - 43	2.76	2.71	0.27	2.71	0.27
1795	14	10.1	13.12	3.14	2.00	2.20	0.22	2.19	0.22
1801	14	10.1	r3.14	3.14	2.27	2.51	0.25	2.43	0.24
1757	17	10.2		3.02	2.29	2.29	0.22	2.27	0.22
1776	16	10.2	12.94	3.10	2.37	2.21	0.22	2.20	0.22
1759	16	10.4	r 2.67	2.93	2.47	2.35	0.23	2.30	0.22
1763	17	10.7	r 3.45	3.44	2.66	2.25	0.21	2.27	0.21
1787 :	•	10.7	12.98	3.17	2.54	1.70	0.16	1.62	0.15
1768	10	10.9	r 3.22	3.33	2.63	1.73	0.16	1.65	0.15
1783	13	11.0	13.43	3.45	2.16	1.96	0.18	2.07	0.19
1784	17	11.0	12.90	3.19	2.40	2.60	0.24	2.48	0.23
1785	15	11.0	12.98	3.16	2.35	2.64	0.24	2.52	0.23
1797	17	11.0	13.32	3 - 53	2.72	2.71	0.25	2.63	0.24
1766	17	11.1	. r3.11	2.45	1.78	2.27	0.20	2.17	0.20
1782	15	11.1	13.06	3.38	2.67	2.22	0.20	2.12	0.19
1781	13	11.2	14.33	4.40	2.09	2.59	0.23	2.35	0.21
1762	16	11.5	r 3.42	3.75	2.79	2.20	0.19	2.22	0.19
1767		11.5	r3.65	3.74	2.72*	2.50	0.22	2.41	0.21
1786	15	12.0	13.45	2.72	2.67	2.67	0.22	2.65	0.22
1770	12	13.0	r4.59	4.44	2.01	2.25	0.17	2.27	0.17
1773	12	13.2	r 5.65	4.99	3.24	2.27	0.17	2.26	0.17
1804	20	13.6	r 4.92	4.89	3.10	2.97	0.22	2.88	0.21
verage						2.20		2.17	

Table 23. The individuals are arranged in order of final cephalo-thoracic length. The specific amount (sp. amt.) of regeneration is the length of regenerated chela propodite per unit of cephalothoracic length.

TABLE 24

Palæmonetes vulgaris. Nos. 1806-1861. Relation of size (age?) to the rate of regeneration. Right chela alone removed

		FINAL		RIGHT	CHELA	AMOUNT OF REGENERA-
	CATALOGUE NUMBER	CEPHALO- THORACIC LENGTH	FINAL LEFT CHELA LENGTH	Original length	Regenerated length	TION PER UNIT OF RE- MOVED CHELA LENGTH
	1831	8.0	. 2.51	2.52	0.52	0 20
Without a molt	1855	9.7	2.75	2.81	0.85	0.30
Without a more	1837	9.7	2 95	2.95	0 79	0.27
	1825	10.0	3.11	3 04	0.70	0.23
	1813	6.1	2.00	1.88	0.60	0.32
With a molt	1849	9.1	2.74	2 62	0.52	0 20
With a mont	1843	11.1	3.77	3.59	0.48	0.13
	1819	11.1	4.07	4.02	0 55	0 14

Tables 24, 25 and 26. The specific amount of regeneration is the regenerated chela propoditelength per unit of removed chela propodite. The short period of regeneration eliminates the probability of error due to change in length of the uninjured chela.

TABLE 25

Palamonetes vulgaris. Nos. 1806–1861. Relation of size (age?) to rate of regeneration

	CATALOGUE	FINAL CEPHALO-	FINAL RIGHT	LEFT	SPECIFIC AMOUNT OF	
	NUMBER	THORACIC LENGTH	CHELA LENGTH	Original length	Regenerated length	REGENERA- TION
	1858	9.1	2.65	2.66	0.73	0.27
	1816	9.3	2.80	2.80	0.77	0.27
Without a Molt	1852	10.5	3.41	3.41	0.83	0.24
Without a Moit	1846	11.1	3.81	3.81	0.82	0.22
	1828	12.1	4.04	4.00	0.69	0.17
{	1840	12.2	4.43	4.52	0.79	0.17
ſ	1834	8.4	2.70	2.64	1.10	0 42
With a Molt }	1810	9.3	2.85	2.88	0.62	0.22
Į.	1822	11.6	3.89	3.68	0.62	0.17

TABLE 26

Palamonetes vulgaris. Nos. 1806-1861. Relation of size (age?) to the rate of regeneration. Both chelar removed

	(ATA-	FINAL CEPHALO-		IEFT CHE	LA	RIGHT CHELA					
	1 OGUE NUMBER	THO- RACIC LFNGTH	Original length	Regen- erated length	Specific amount	Original length	Regen- erated length	Specific amount			
	1854	8 4	2 56	0 74	0 29	2.56	0 74	0.29			
	1809	8 9	2 62	0 59	0 23	2 62	0.59	0.23			
	1857	90	2.44	0 62	0.25	2.52	0.66	0 26			
	1836	9.1	2 69	0 59	0 22	2 69	0 59	0 22			
	1824	10 4	3 12	0 81	0 26	3 14	0.89	0.28			
Without	1851	116	3 35	0 83	0 25	3 35	0 89	0.27			
à	1861	11.7	3.48	0 85	0 24	3.48	0 81	0 23			
Molt	1818	118	3 92	0 94	0.24	3 92	0.90	0.23			
	1827	11 9	3 55	0.62	0 18	3 55	0 56	0.16			
	1845	12.1	4 24	0 84	0 20	4.24	0 93	0.22			
	1839	12.7	3.96	o 77	0 19	3.96	0 77	0.19			
	1812	6.5	1 93	0.76	0 39	1 93	>	>			
	1860	7.9	2.37	1 26	0 53 .	2.37	1 36	0 57			
	1806	8.3	2.48	0 79	0.32	2.48	0.80	0 32			
With	1833	9 2	2 89	0.52	0.18	2 90	0 52	0.18			
a	1830	98	3.06	0.65	0.21	3.13	o 68	0.22			
Molt	1842	10 0	3 11	1.48	0.48	3.11	1 48	0 48			
	1821	10.0	3.40	1 41	0.41	3 40	1 37	0.40			



# CONTRIBUTIONS TO THE PHYSIOLOGY OF REGENERATION<sup>1</sup>

## I EXPERIMENTS ON PODARKE OBSCURA

RY

#### SERGIUS MORGULIS

#### WITH SEVEN TEXT-FIGURES AND THIRTY-THREE TABLES

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### I PRELIMINARY STATEMENT

It is, perhaps, a truism to say that the regeneration of an organ or of a portion of an organism is the function of the organism as a whole rather than of the injured surface alone. Even on purely a priori grounds this would appear to be a reasonable and physiologically justifiable point of view. To prove this beyond a doubt would, however, be exceedingly difficult, the difficulty arising from the peculiarity of the problem. Wherever there are two

<sup>&</sup>lt;sup>1</sup> This work was started and carried on for two summers at the Marine Biological Laboratory, Woods Hole, Mass., and it is a pleasure at this occasion to express my gratitude to the officers of the institution for the many courtesies shown me while there. To Dr. F. R. Lillie, the Director of the Laboratory, I am specially obliged for his kindness in affording me the opportunity to pursue this investigation. My thanks are also due to Dr. E. L. Mark for reading the first draft of this paper.

factors involved in bringing about a certain reaction, the significance of each may be judged by the modification of the reaction occasioned by either changing or completely eliminating one of these factors. This method could not be resorted to, to decide the question whether the whole injured organism or the injured surface alone is concerned with proliferation and growth of tissue for the reason that we can neither eliminate the organism and retain the cut surface, nor retain the organism without the cut surface. A definite solution of this problem is, nevertheless, quite important for the proper understanding of the process of regeneration.

We might, of course, point to the fact that regeneration is accompanied by changes in the equilibrium of the injured animal form, as was brought out by Przibram ('07), and that the regeneration from the cut surface is sometimes concomitant with phenomena of reduction or compensation affecting non-injured and distant parts of the body (Kammerer '07).

It is true that the histogenetic phenomena are specially pronounced near the cut end, but, as was shown in the regenerating hydra—"new cells are not found at the cut surface alone. Divisions were found to occur as well at the sides as at the ends of the regenerating piece." (Rowley, p. 582.)

Furthermore, if regeneration is a function of the organism, it should be open to modifications contingent upon changing the physiological condition of the organism; and a part of the present paper deals with this problem. Additional evidence, derived from the study of the regeneration in Lumbriculus, will be presented in the second paper of this series.

Acknowledging the pertinence of objections that may be raised and admitting the great difficulty of establishing by circuitous ways beyond further dispute the view which was put forth at the beginning, it seems none the less a cogent argument that an accumulation of facts which indirectly bear out this hypothesis may swing the balance strongly in favor of the view, which from the physiological standpoint is the more probable.

## II RATE OF POSTERIOR REGENERATION

This marine polychæte (Podarke obscura) is composed usually of 40 to 50 segments. The dorsal surface is, as a rule, covered by a chitinoid layer, which varies in color from very dark brown to a light shade of yellow. In a few exceptional cases the worms are devoid of this covering, appearing then to be "naked," and are quite transparent.

The worms possess the power of posterior regeneration only; as far as could be ascertained, they never regenerate a new head. Normal and perfect regeneration will take place from an exposed surface produced by the animal itself, which is able through the contraction of the dermal muscles to detach a larger or smaller number of its posterior segments. Such a condition can be very easily reproduced artificially in the following way. The animal's back is pressed down gently with the flat blade of a scalpel near the region where the separation is desired. In response to the mechanical stimulation the worm will attempt to crawl away, but since a portion of its body is held down by the scalpel, it will not be able to do so. A vigorous contraction will then occur resulting in a constricting off of the free portion from the rest of the worm's body. By this method it is possible to cause even single segments to be pinched off very readily, and the experimenter is thus enabled to control the number of segments in the piece retained for the experiment. The wound formed through such a reflex contraction of the musculature will soon close over, and the regeneration following upon such an operation is invariably perfect. In those cases, however, where the cut has been made with a knife, and the mutilated segment has not been thrown off by the animal afterward, as often happens, the new tail will show abnormalities either in size or the direction of its growth, or in both.

As mentioned above, it is possible to remove as many segments as desirable with a great degree of accuracy. Besides, Podarke also furnishes the opportunity of keeping an exact record not only of the number of segments removed, but also of those remaining in the old piece, as well as of the regenerated segments, since the

numbers are rather small and the segments can be easily counted owing to their large size and to the presence of paragonia. In the experiments to be described in the following paragraphs a complete record has been kept, with the object in view of determining in what relation the regenerated segments stand to the old and to the removed segments.

A number of worms were divided into three groups. In the first group (A), containing 23 worms, 471 segments in all were removed; that gives an average of about 21 segments (20.5) to each individual worm. The total number of segments left over in the old pieces of the worms amounted to 381, or an average of about 17 segments (16.6). Thus in the first group on the average a little over one-half of the worm's body was cut off. In a second group (B), containing 19 worms, 273 segments were removed, or on the average about fourteen (14.4), while the number of segments in the pieces experimented on was 441, or an average of about twenty-three (23.2). Thus, in this group a little over one-third of the worm was cut off. Lastly, in the third group (C), containing 14 worms, where only 111 segments were removed (8 on the average), and 425 (or about 30 for each piece) were retained, only about one-fourth of the worm was taken off.

The worms were kept in pure sea-water, in which there was no food present.

In Table I are given the average numbers of old segments and removed segments as well as of segments regenerated in the course of two weeks, likewise the corresponding data for each individual worm.

From this table we learn that 155 segments (an average of 6.8) have been regenerated from the pieces of worms of the first group; 120 segments (an average of 6.3) from those of the second group; and 68 (4.9 on the average) from pieces of the third group. These facts are expressed graphically in the diagram shown in Fig. 1.

The ratio between regenerated and the original anterior segments expressed in percentages is 40.7 for Group A, 27.2 for Group B, and 16 for Group C. The ratio between the number of regenerated segments and of those removed expressed in per-

TABLE I
July 20 to August 4 (1907)

	Α			В		C					
NUMBER OF SEG- MENTS REMOVED	NUMBER OF OLD SEGMENTS	NUMBER OF RE- GENERATED SEGMENTS	NUMBER OF SEG- MENTS REMOVED	NUMBER OF OLD SEGMENTS	NUMBER OF RE- GENERATED SEGMENTS	NUMBER OF SEG- MENTS REMOVED	NUMBER OF OLD SEGMENTS	NUMBE OF RE- GENER ATED SEG- MENTS			
23	14	3	15	23	3	7	4 <sup>1</sup>	3			
19	19	3	13	26	4	9	36	3			
18	19	3	15	33	4	10	25	4			
20	16	4	16	24	5 ,	6	37	4			
20	16	5	16	25	5	8	29	4			
20	16	5	15	22	6	6	23	5			
24	14	5	1 15	17	6	8	31	5			
20	. 15	5	13	. 27	6	9	30	5			
22	16	6	16	26	6 6	<b>8</b>	32	6			
20	21	6	13	24 . 23 .		8 7		6			
22	16	7	16				31	6			
20	17	7	15		7	10	28	7			
19	18	7	15	20	7	8	26	7			
23	18	7	14	22	7	7	29	3			
23	18	. 7	13	26	7	•	1				
20	12	1 8	15	23	8						
20	15	9	13	21	9						
18	14	9	10	20	, 9						
20	20	9	15	19	9						
16	18	. 9	• ,	-9	, ,						
18		10	 	1 <b>1</b>	·						
22	14	10	İ	İ	;						
	15 20	11									
22	20				<u> </u>						
			A	verage Num	bers			_			
20.5	16.6	6.8	14.3	23.2	6.3	8	30.4	4.9			
		R	Ratio of new	to old segme	ents in per co	nt	Total State				
	40.7	i		27.2			16				

centages is 32.9 in the first group, 47.6 in the second group, and 61.3 in the third group.

These ratios between regenerated and original segments, and between regenerated and removed segments are almost precisely in inverse proportion, in so far as they present a descending and an ascending series. The question, then, naturally arises as to which of these two series, the descending or the ascending, is the real expression of the rate of regeneration at the three different levels.

If we take as a basis of comparison of rates of regeneration at different levels the extent to which the removed parts are restored, we shall have to admit that the highest rate of regeneration (resto-

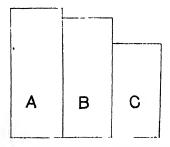


Fig. 1 Shows the extent of regeneration from three different levels.

ration) is found in the most posterior part of the worm, and that this gradually decreases as we advance towards the more anterior regions. As a matter of fact, in the third group of worms (C), where about one-fourth of the animal's body had been removed, more than half of the removed segments were restored; while in the second group (B), where over one-third of the number of segments had been cut away, less than one-half; and in the first group (A), where half of the segments had been cut away, only one-third of the number of removed segments were regenerated.

If, on the contrary, we assume as the basis for comparison of the rates of regeneration at different levels the amount of new tissue produced for each unit of retained old tissue, it will be found that, whereas the anterior region will regenerate on an average about 41 per cent of itself, the middle and posterior regions will regenerate only 27 and 16 per cent respectively.

The latter basis is undoubtedly the correct one, since it is obvious that when a small piece regenerates a relatively larger tail than a larger piece does, the cell-activities underlying all the vital phenomena of the organism must be greater in the former case than in the latter. It is this vital activity, the metabolic processes of the individual cells, which, on this view, must determine the rate of regeneration. This physiological explanation is rendered probable by the very existence of such a thing as different rates of regeneration, and though largely speculative as yet, it opens up a promising field for further experimental work.

#### III PHASES OF POSTERIOR REGENERATION

From experiments performed during the summer of 1907, I arrived at the following conclusions: first, that there is invariably a lapse of some time before the new tail begins to regenerate, this period being followed by a proliferation of new, undifferentiated tissue; secondly, that new segments with definite structural characters are formed out of this undifferentiated material, and that this formation of new segments takes place at the highest rate somewhere between the fourth and the twelfth day after the operation; thirdly, that a sudden rise in the rate of regeneration alternates with a sudden diminution in the rate of production of new segments, the increments becoming continually smaller and smaller till finally no more new segments are formed; and lastly, that, although fewer new segments are being added, the segments already formed are increasing in size. These inferences were drawn from a frequent (usually daily) examination, which extended over nearly seven weeks, and was made on worms cut in two near the middle of the body.

This experiment gave the starting point for some further experiments which I undertook in the summer of 1908.

The principal problems to be solved were these: First, At what stage of the process of regeneration at different levels does the difference in the rate of regeneration first appear, and how is this difference maintained throughout the later stages? Secondly,

What are the exact relations between the process of the formation of new segments and the process of the growth of those segments?

With these questions in mind a number of worms were divided into two lots. In one lot about two-thirds of the body was removed, while in the other lot one-third was cut off. The worms were examined every four days and sometimes at intervals of two days. Records were kept of the number of regenerating segments and of the length of the tails measured by a compound microscope with eye-piece micrometer.

The experiment was started on August 10, 1908, and within twenty-four hours after the operation the edges of the wound were closely drawn together, but there was as yet no trace of new tissue. On the second day, however, many of the worms had already produced new tissue. But in this respect there was an important difference in behavior between the worms from which two-thirds of the body had been removed and those from which only one-third had been removed. From the records made on August 12, it was found that 50 per cent of the former but only 14 per cent of the latter had proliferated new material.

At this stage one finds only a bud of undifferentiated new tissue, which grows out through a small foramen in the region of a coalescence of the margins of the wound. This bud of regenerated material takes on the appearance of a plug. In the course of the succeeding days this plug of tissue increases not only in length, but likewise in its circumference, producing thus a reopening of the margins of the wound previously drawn together. There is very little differentiation in the proliferated tissue, though a beginning of it can already be observed. In Fig. 2 are represented a few buds found on the second day; these as arranged form a complete series of stages of regeneration for the first two days.

At first the plug of new tissue has a perfectly round surface (Fig. 2, a), but then occurs an increased lateral growth (C), which results in the formation of two horn-like elevations. On the second day in some worms these elevations may become separated from the rest of the proliferated material by delicate septa (e); ultimately they will grow into long brittle-like appendages of the anal segment.

Next, the anal segment becomes differentiated and assumes its characteristic dome-shaped form. Then other segments also differentiate out of the already proliferated material. The new segments when first differentiated are very short, and can be detected only under a compound microscope. Soon, however, they acquire a sharp outline and at about this time there appear two localized outgrowths, one on the right and one on the left. These minute outgrowths foreshadow the future parapodia. The finer details of the anatomical structure of the segments develop gradually as the whole segment increases in size.

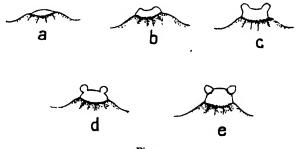


Fig. 2

At the end of four days in worms, from which two-thirds of the body was removed, there are already from two to four new segments regenerated. These differ from the normal, old segments only in size and more particularly in the simpler structure of their parapodia.—Compare Table II.

TABLE II

August 10 to August 14													
													Average
No. of old segments	17	18	18	15	19	18	17	16	19	17	16	15	17
No. of new segments	2	2	2	2	3	3	3	3	4	4	4	4	3

After a lapse of four more days, i. e., eight days after the operation, the number of regenerated segments varies from 3 to 6, but worms with 6 regenerated segments are by far the most common.

On the 16th day the rate of regeneration varies within still wider limits, but those with eight regenerated segments are quite typical.

The considerable degree of variation in the number of regenerated segments at the period of 12 to 20 days after the operation is due to the fact that some of the worms are slower in starting to regenerate and which also linger behind the rest of the worms in the process.

The records of the number of new segments found at the end of every four days, likewise the average number of segments for each corresponding period, are given in Table III

						TAI	BLE	Ш							
-	DATE OF		No. of Regenfrated Segments												AVERAGE LENGTH OF RF- GEN. TAILS
Aug.	18	! "	4 4 5 6	5 5 6 6	5 6 6	6 7 7 8	6 8 7 8	6 ! 8 ! 8 !	6 8 9	6 8 9   9	6 9 9 1	9 10	10	5·3 6.6 7·4 8.1	0.42 0.69 0.84
Sept.	3:	6	6	7	٠7	8	8	9	9	9	1		11	8.3	1.00

TARTE TIT

From this Table III it will be seen that after a period of rapid formation of new segments (the first four days) the number of segments regenerating for a certain period of time begins to decrease and continues to do so steadily. This condition is illustrated in the diagram, Fig. 3, where the horizontal line is divided into equal portions, each corresponding to a period of four days, and the vertical columns correspond to the average number of segments regenerated in course of those periods, as shown in Table III.

Of the worms from which one-third of the body had been removed, only 14 per cent proliferated new tissue at the end of two days; for the first four days from 2 to 3 new segments have regenerated, as seen from Table IV.

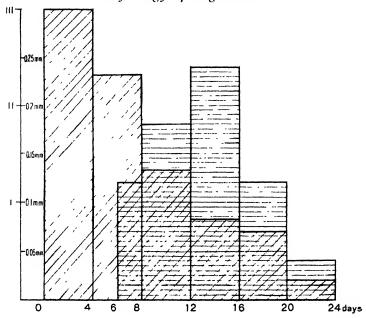


Fig. 3 The obliquely striated columns represent the *number* of segments regenerating for equal periods, of four days each, at the anterior level. The horizontally striated columns represent the increments in the *length* of the regenerating tails for the same periods of time. The Roman numerals refer to the number of regenerated segments, while the Arabic numerals to the length of the regenerated tails in mm.

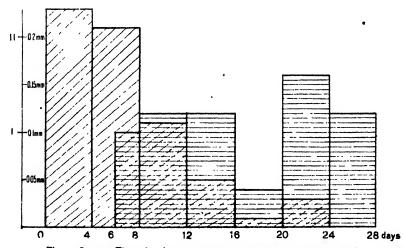


Fig. 4 Same as Fig. 3, but for worms regenerating at a more posterior level.

TABLE IV

		Aug	ust I	O to	Augu	st 14								
	i			[ 		Ī							Average	
No. of old segments  No. of new segments	30	28	31	30	32	33	30	30	33	27	25	30	30	
No. of new segments	2	2	2	2	2	2	2	2	2	3	3	3	2.3	

After 8 days from 3 to 5 segments have regenerated, and from 4 to 7 segments after 12 days, etc. The records are presented in Table V.

	TABLE V		
DATE OF EXAMINATION	NO. OF REGENERATED SEGMENTS	AVERAGE NO. OF SEG- MENTS	AVERAGE LENGTH OF RE- GEN. TAILS
Aug. 18	3 3 4 4 4 4 5 5 5 5 5 5 5 5 5 5 5 6 6 6 6 6	4.4 5.5 6.0 6.1 6.8* 6.8*	mm. 0.32 0.44 0.56 0.60 0.76 0.88

<sup>\*</sup>This high average was caused by the fact that most of the worms with a small number of regenerated segments have died. With the aid of individual records of the worms it was possible to ascertain definitely that only four new segments were added since Aug. 30, so that the real average is but 6. 4. In constructing the diagrams I used this average as the correct one.

From this table it will be seen that what has been said with regard to the gradual diminution of increments of new segments after a period when these increments were quite considerable, in the case of worms from which two-thirds of the body had been removed, holds true also in this case. The accompanying diagram, Fig. 4, constructed on the same principle as the previous one, will illustrate the point.

It is thus seen from the preceding tables and diagrams that the rate of forming new segments diminishes as the time after the operation increases. Unfortunately the worms of this experiment

died before the completion of the process, but I know from the results of other similar experiments that, while at the end of 36 days the number of regenerated segments may reach eleven or twelve, hardly any more new segments will be found at the end of forty-five days. The process, then, has not merely been slowed down, but practically brought to a standstill by that time.

If, now, we compare the results of the process of regeneration from the two different levels, we find, in the first place, that the number of worms which proliferate new tissue soon after the operation is four times as great in case of worms from which nearly two-thirds of the body are removed than in the case of worms from which only about one-third of the body is cut off. This difference is of some theoretical importance since it reveals the fact that the reaction takes place much more vigorously at one level than it does at the other, and therefore a greater activity must have ensued as the result of the operation in the worms cut nearer the head than in those cut near the tip of the tail. We shall find, furthermore, that already at the end of the first four days after the operation worms regenerating from a more anterior level are in advance of those regenerating from a more posterior one, and that this condition is maintained practically throughout the entire period of regeneration. In the diagram, Fig. 5, this point is illustrated by superimposing the diagram, Fig. 4, over the diagram, Fig. 3.

This diagram makes it perfectly clear that there is not merely a difference in the rates of regeneration from two different levels at some particular stage in the process, but that there is, so to speak, a continuous and lasting difference. This cannot be due to a more effective slowing down of the regenerative process in one case than in the other, as in either case the regenerating tail goes through a similar cycle of stages: a short interval of no apparent activity soon after the operation, then a very sudden rise in the regenerative activity followed by a sudden drop in the process, which is on the decline from this time until it reaches practically a standstill. But there is a constantly greater output at one level than at the other, as is illustrated by the diagram, Fig. 5, in which the obliquely striated columns correspond to the

average number of segments regenerating for equal periods of four days from the anterior level, while the horizontally striated columns are representative of the numbers of segments regenerated from the posterior level. In accordance with our present state of knowledge it seems fair, therefore, to assume that the greater output throughout the whole process of regeneration indicates a condition of higher physiological activity in one case than in the other.

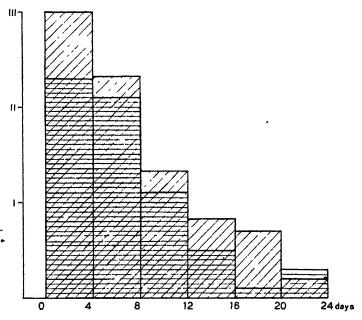


Fig. 5 This is to show the relation between the rates of regeneration from two levels during the regenerative process. The obliquely striated columns represent the rate of regeneration from the anterior, while the horizontally striated columns represent the rate of regeneration from a posterior level, for similar periods of time.

Now, from a study of posterior regeneration what can be learned concerning our second problem, which, as stated in the beginning of this chapter, is to ascertain the exact relationship between the process of formation of new segments and their subsequent growth?

When we examine regenerating tails about the sixteenth day after the operation, we usually find them as yet very small; in

fact they can easily be overlooked on superficial inspection. But the same regenerating tails at the end of 35 to 45 days will have already attained a large size, and worms from which but few segments were removed can hardly be distinguished from normal ones. During those 20 to 30 days very few segments were added, but what really did take place was a considerable increase in size of each of the regenerated segments.

In 1908 I kept a record of measurements of the regenerating tails made at regular intervals. The tails were always measured in an extended condition. The data of the average length of the tails at various periods of their regeneration are presented in the right-hand columns of Tables III and IV.2 Glancing over these data it can be seen at once that for a time they form a progressive series, i.e., the increments for successive, equal periods are growing larger. In diagram, Figs. 3 and 4, these data are represented graphically by the horizontally striated columns. The horizontal units indicate the number of days (four days to each column or period), while the height of the columns represent the increment in mm. for the given periods. One-hundredth of a millimeter of the actual length of the regenerating tail is represented by o.1 of an inch in the diagram. The horizontally striated columns are superimposed upon the obliquely striated ones in such a way that the average increment in the number of segments (obliquely striated columns) and the average increment in the length of regenerating tails (horizontally striated columns) for the same periods of time coincide with one another. It will be readily seen that while the former present a retrogressive series, the latter present (at least in some portions of the total period) a progressive series. Thus it appears as if we were dealing here with two phenomena, antagonistic to each other in the sense that while one is going on with great and sometimes, increasing intensity, the other is gradually diminishing. These two phenomena are: formation of segments from the proliferated tissue, and growth or increase in size of those segments.

<sup>&</sup>lt;sup>2</sup> The average lengths of tails on the sixth day after the operation (August 16) are not given in these tables. They were 0.30 mm. for tails regenerating from anterior, and 0.22 mm. for those regenerating from the more posterior level.

## IV RATE OF REGENERATION AFTER SUCCESSIVE OPERATIONS

Does an organism regenerate faster or slower after it has been operated upon for a second or a third time? This seems to be an important point in the study of regeneration at the present time. In an earlier publication (Morgulis '07) I demonstrated that in the case of Lumbriculus the rate of regeneration decreases approximately geometrically after successive operations from the same pieces of worms, the time element (14 days) remaining the same in all three cases. The indices of the rate of regeneration from pieces of three different levels were respectively 3.1, 2.6 and 1.8 after the first operation; 1.8, 1.5 and 1 after the second operation; and 0.8, 0.6 and 0.5 after the third operation.

In a paper, which appeared somewhat later, Zeleny ('08) states that in the scyphomedusan Cassiopea xamachana the reverse is true, the rate of regeneration there being greater after a second operation.

In some recent experiments ('08) on regeneration in the scarletrunner bean, I performed an experiment for the purpose of determining the rate of regeneration after a second operation, and there, again, I found that not only is the interval of time intervening between the operation and the first appearance of regenerating stems greatly prolonged, but also the regenerating stems themselves are considerably smaller than those regenerated after the first operation, the weight of the regenerated stems and the index of transpiration being also most decidedly lower after the second operation.

During last summer (1908) I studied again the rate of regeneration after successive operations in Podarke. The worms were divided into two groups. In one group the number of segments regenerated during six days was ascertained and the regenerated tails then cut off. In the other set the regenerated tails were cut off at the end of twelve days from the time of the operation. In the first set of worms there had regenerated in six days 4 to 5 segments (4.4 on the average). These new tails were then severed from the body, and the worms were again examined at the close of six days. The unfavorable effect of the second operation upon

the rate of regeneration was striking. This time only one-half the number of worms had regenerated at all, while the number of segments in the regenerated tails was only two. This will be seen from Table VI.

TA		VI

			•						-		,
AUGUST 10-16, 1908	i i	NUMBER OF SEGMENTS.								AVERACE	
Old segments	. 18	20 1	16	18	14	18	17	16	17	19	17.3
New segments	. 4	4	4	4	4	4	5	5	5	5	4.4
Regenerated					_		-	-	-		
Aug. 16-Aug. 22	. bud	bud	0	٥	2	2	2	2	2	3	2.1
Aug. 16-Sept. 3	. 5	7	8	9	i						

There was thus a most marked decrease in the rate of regeneration for the second period of 6 days as compared with that for the first 6 days.

In the other set (see Table VII) the worms were allowed to regenerate for 12 days, at the end of which period they had regenerated on the average a little more than five segments. The tails were cut off once more and the worms left to regenerate anew. They were examined at the end of twelve days. Here again it was found that the rate of regeneration suffered a considerable reduction after the second operation, and though the length of time during which the worms were regenerating was the same in both cases, the number of regenerated segments was on the average greater by 1.5 segments after the first operation.

TABLE VII

AUG. 10-AUG. 22		NUMBER OF SEGMENTS									AVERAG			
Old segments New segments	17	20	15	21	18	17	20	21	15	19	21	21	20	19 5·3
New segments	-	2	1		_ ا	ے ا	-	_ ا	1 6	· _		:		
		!	<u></u>		!			<u> </u>	1	'	<u>'</u>	7		3.3
		!	<u></u>		nis re			<u> </u>	1	! 7	<u>'</u>	7	•	3.3
	Reg	enera	ted s	egme	nis re			<u> </u>	22			7	•	3.3
August 22 to September 3	Reg	enera	ted s	egme	nis re			<u> </u>	22	dead		/	•	3.8

When, however, the worms of these two groups are allowed to regenerate for a longer time, we find a rather interesting condition. In the first place, it will be found that the retarding effect produced by the second operation wears off in the course of time, and the worms regenerate again at a nearly normal rate. Moreover, it will be observed that the ones which had regenerated the smaller number of segments before the second operation was executed required a shorter time to regain the normal rate of regeneration than those which had regenerated a larger number of new segments when operated on the second time. From Tables VI and VII it will be seen that while the worms of the first set (Table VI), at the end of about eighteen days, regenerate at a nearly normal rate, those of the second set (Table VII) are still under the influence of the second operation and regenerate (on an average) a smaller number of segments than normally.

It will also be seen from Table VII, that while after the first operation from 3 to 8 new segments had been regenerated by the end of twelve days, after the second operation even during a period one and one-half times longer the same worms had regenerated only 5 to 6 new segments.

It would be interesting to determine by special experiment the relation that exists between the period at which the second operation is performed and the immediate effect of the operation upon the rate of regeneration, as well as the after-effect of the operation.

### V RELATION BETWEEN FOOD-SUPPLY AND RATE OF REGENERATION

The rôle of food in regeneration is commonly supposed to be of slight or of no importance, but the following experiments will show that such an attitude toward this factor is not warranted. There are on record some unquestionable cases, in which no definite relation between food-supply and regeneration could be found; as, for instance, that of the splendid experiments of McCallum ('05) with plants, from which he arrived at the conclusion that "there need be no increase in nutritive conditions

to occasion regeneration," or those of Morgan ('01-'02) in which he showed that planarians in the last stages of starvation will still draw upon their emaciated bodies to make good a portion removed by an operation.

These are, indeed, very striking cases; nevertheless it is going too far to maintain on the basis of these data that food usually has no effect whatever.

In the present chapter we are concerned with the food-supply not as a factor in regeneration, but rather as a possible factor in determining the *rate* of regeneration. The following data have been derived from experiments which were conducted during the summer of 1907 and repeated in 1908.

A number of worms were separated into three groups. In the first group  $(A_1)$  the total number of segments removed was 171 (or 21.4 on an average), and that of the segments left in the pieces experimented on was 135 (an average of 16.9). The same numbers in the second group  $(B_1)$  were respectively 127 and 180 (or on the average 14.1 and 20 segments respectively). In the third group  $(C_1)$  these numbers were respectively 83 and 249 (an average of 9.2 and 27.7).

This experiment was carried out as has been stated in 1907, and the data presented in Table I (p. 599) are to serve as the control. I may also say that the worms used in the experiment and in the control belonged to the same lot, all having been collected from one place and at the same time.

The average numbers of segments removed and of those present in the pieces used in the experiment (A<sub>1</sub>, B<sub>1</sub>, C<sub>1</sub>) are almost identical with the corresponding numbers of the control (A, B, C).<sup>4</sup>

The worms of the three groups were kept in separate dishes and were provided with the sea-weed, on which they are always found, taken from the Woods Holl "Eel-pond." Thus, unlike the worms of the control they were constantly provided with food.

<sup>&</sup>lt;sup>8</sup> It has, however, been shown recently by Kupfer ('07) that regeneration in plants is dependent upon an adequate supply of food; that in plants from which the reserve food has been exhausted no regeneration takes place and that also in white shoots of several species the same holds true.

<sup>&</sup>lt;sup>4</sup> It will be recalled that the worms of this experiment were kept in dishes with pure sea-water and were deprived of any food.

In Table VIII are recorded the number of segments removed, remaining and regenerated; the average of each and the ratios of the regenerated to the old segments remaining after the operation.

TABLE VIII
July 23 to August 7 (1907)

		A,			$\mathbf{B_1}$			$C_i$	
	NUMBER OF SEGMENTS REMOVED	NUMBER OF OLD SEGMENTS	NUMBER OF REGENERATED SEGMENTS	NUMBER OF SEGNENTS REMOVED	NUMBER OF OLD SEGNENTS	NUMBER OF REGENERATED SEGMENTS	NUMBER OF SEGMENTS REMOYED	NUMBER OF OLD SEGMENTS	NUMBER OF REGENERATED SEGMENTS
	21	15	5	15	25	4	9	30	4
	20	19	6	11	26	4	10	30	4
	22	18	9	15	23	7	9	30	5
	21	16	9	II	20	8	9	30	, 5
	23	17	10	17	20	9	9	27	7
	20	17	11	16	15	9	9	24	7
1	23	18	11	17	18	10	10	27	8
	21	15	12	14	19	10	9 '	24	8
				11	14	11	9	27	9
Average no. of segments	21.4	16.9	9.1	14.1	20	8	9.2	27.7	6.3
Ratio of new to	54	.ı per ce	ent	4	o per cei	nt	22.	9 per ce	nt

Expressing these ratios in percentages they are, for the worms of the first group, 54.1; for those of the second, 40, and for those of the third group, 22.9. Comparing these results with similar ratios between old and regenerated segments found in Groups A, B and C of the control (see Table I) it is evident that there is an increase in the number of regenerated segments in each of the three groups. The increase amounts to nearly 14 per cent in the first group, nearly 13 per cent in the second group, and nearly 7 per cent in the third group. Therefore, it may be said that on the average for all three groups to every 100 old segments in fed worms approximately 11 more new segments were regener-

ated than in the control worms, which were kept in pure seawater.

I performed the same kind of an experiment in the summer of 1908 with the same result. As will be seen from Table IX, the old pieces were practically of the same length in the control and in the experiment. But there was a considerable difference in the number of segments regenerated from worms that had been fed and those that had not been fed.

TABLE IX

						-	-							
AUGUST 12-AUG	NUMBER OF SEGMENTS										AVERAGE			
Worms not fed	old segments 22 new segments 4	17 15 1 4, 4,	8 18 4   4	201	5 16 19 5 5 5	14								17.4 4 6
Worms fed	old segments 23	15132	5 6	201	6 17 17 6 6 6	18 I 6	617:	7	9 16 1 7 7	7 7	20 I	9 15 1 7 7	7, 8	21.3
			•	rab'	LE X				_					
AUGUST 22-AUG	ust 28, 1908	1			NUM	RER	OF	SEG	MEN	r8				AVERAGE
Worms not fed	new segments	4	4	5	5 6	8				i		i	,	5.3
Worms fed	new segments	6	6	7 ,	7   8	9	9	9	9	9	9	10 1	0 10	8.4

From this table we find that at the end of ten days after the operation the number of regenerated segments of the control worms varied from 4 to 6, while that of the well-fed worms varied from 4 to 8. A similar difference was found with the same lots at the close of sixteen days (see Table X), when the number of new segments varied in the control from 4 to 8, and in the experiment from 6 to 10.5 The mere numbers do not give a perfect presentation of these differences, which are by far more striking when the animals themselves are compared. From exact meas-

<sup>&</sup>lt;sup>8</sup> Unfortunately some of the control worms died before the end of the experiment. This accounts for the fact that the number of specimens in the control is smaller than in the experiment. In view of this circumstance I wish to point out that in 1907 there were twice as many specimens in the control (Table I) as in the experiment (Table VIII). The results, however, are similar in both cases.

urements of the regenerating tails by means of the eye-piece micrometer it was found that at the end of sixteen days the tails of the fed lot had grown to almost twice the length of the tails of the control worms, which had been deprived of food.

Should we, now, compare the rates of regeneration from the three different levels in worms that were fed and in those that were not (compare Fig. 6), we shall not fail to be impressed with the fact that in both cases the rates of regeneration from the different levels form a descending series; but what is still more interesting and important is that in well-fed and in starved animals, i. e., when there is a higher or a lower rate of regeneration, the ratios between the rates of regeneration from different levels remain practically the same.

The problem of the relation between food-supply and the rate of regeneration from different levels was taken up by certain investigators with special reference to Zeleny's tentative hypothesis, viz: that the differences in the rates of regeneration of arms in the brittle-star Ophioglypha lacertosa might perhaps be due to the different amounts of food available for the regenerating part in animals with one, two, three or four arms cut off. It has since been shown, however, by Morgan ('06) and by myself ('07) that even in starved animals there is a difference in the rates of regeneration from various levels, thus rendering food-supply at least a secondary factor, if indeed it has any influence in determining the rates of regeneration from different levels.

The evidence previously adduced may be considered as merely negative. That which the present experiments offer is, I believe, of a more positive kind. Though food is a factor determining to a certain extent the rate of regeneration of an organism, it is not the factor which determines the difference in rates of regeneration from different levels. I desire, therefore, to call special attention to this distinction in the influence which is attributed to the factor of food in regenerative processes.

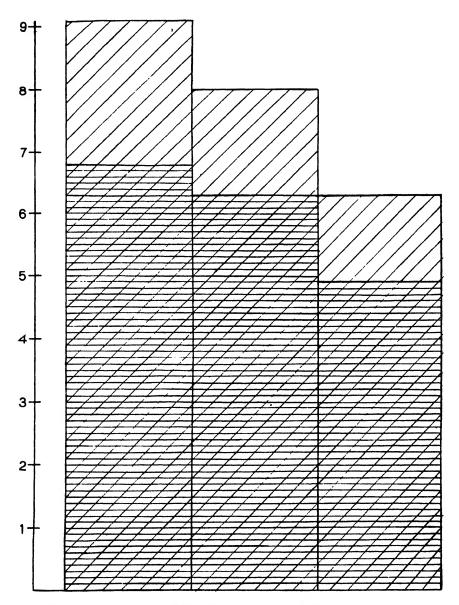


Fig 6 This diagram gives a comparative view of the results from worms cut at three different levels. The horizontally striated columns represent the number of segments regenerated by starved worms (Table I, A, B and C); the obliquely striated columns represent the number of segments regenerated for the same period of time and from a corresponding level by fed worms (Table VIII, A, B and C).

# VI STIMULATION AND DEPRESSION OF PROTOPLASMIC ACTIVITY AS DYNAMIC FACTORS IN REGENERATION

The present chapter is based upon experiments which were performed in 1907, and then repeated in 1908. It will be convenient to give first the results of each experiment of 1907 and then the results of the corresponding experiment performed the following year.

If regeneration is a function of the entire organism, it must be dependent upon the physiological condition of the organism, and should, therefore, be subject to acceleration and retardation on altering the physiological condition. This hypothesis was put to the test of experimentation, of which the following chapter is the outcome.

# First Series of Experiments

In this set of experiments was tested the effect of alcohol and chloretone.

# Experiments with Alcohol

A large number of worms cut anteriorly to the middle of the body were subjected to the action of a solution of alcohol and sea-water, in the concentration of 1:25,000. The treatment extended over one-third of the time of the experiment only and, although none of the worms regenerated more segments than the worms with the largest number of new segments of the corresponding control, the effect of the alcohol was very definite. As compared with the control, there was a great preponderance of worms with large numbers of regenerated segments in the alcohol solution. A similar experiment was done in 1908, but this time the worms were kept in the alcohol solution during the entire period of their regeneration. The results were more positive as will be seen from the data given in Table XI.

The middle column contains the data for the control, the right and left columns contain those of experiments with alcoholic solutions of 1:10,000 and 1:20,000 concentration respectively. The number of regenerated segments in the control varies from

TABLE XI

Effect of Alcohol

August 21 to August 31 (1908)

measure energetimes actions decreases units in deep life. An in	1	-	ī	-		+	
ALCOHOL IN SEA-W.	1	CONTROL (SEA-W.)	1		,	AL	COHOL IN SEA-W.
1:10,000	1		İ				1:20,000

Number of segments

Old	Regenerated	Old	Regenerated	Old	Regenerate							
18	2	16	3	14	4							
16	3	18	4	16	4							
17	3	17	4	18	4							
16	4 1	16	4	19	4							
16	4 '	18	4	18	4							
16	4	15	4	17	5							
18	4	18	4	16	5							
18	4	15	4	17	5							
18	4	18	4	20	5							
		17	5	15	5							
				16	5							
				17	5							
				18	5							
				17	6							
7. 17	3.6	16.8	4	17	4.7							

3 to 5, while the same varies in the strong solution of alcohol from 2 to 4, and in the weaker solution from 4 to 6. The inhibiting effect of the stronger solution and the stimulating effect of the weaker upon the rate of regeneration are obvious from the experiment. But the difference in favor of those worms that were subjected to the influence of the weaker alcohol could be a good deal better appreciated by actually examining the animals than by merely counting the number of segments. Two weeks later the worms were inspected once more and it was found that while those in the control had regenerated from 7 to 9 new segments, those in the weaker solution of alcohol (1:20,000) had regenerated from 8 to 11 new segments.

It is known to physiologists that alcohol in moderate doses augments the activity of protoplasm, and the results of these experiments conform in general with this physiological fact.

# Experiments with Chloretone

Chloretone has been made much use of within recent years to stupefy or simply to quiet animals. The addition of a few drops of a weak solution of chloretone to the water in which an animal is kept is often sufficient to slacken its movements. It seemed desirable to investigate the effect that chloretone would produce upon a regenerating animal. A 0.001 per cent solution of chloretone in sea-water was employed in this experiment. The regenerating worms were submerged in the solution for one-third of the entire duration of the experiment. The effect of this treatment will be easily observed by examining Table XII.

TABLE XII

Chloretone 0.001 per cent
July 26-August 10 (1907)

Removed	Old	Regener- ated	Removed	Old	Regener- ated	Removed	Old	Regener ated
.28	20	3	21	14	4	20	16	6
19	17	3	20	17	4	19	19	6
23	17	3	20	17	4	23	16	7
23	15	3	20	16	4	22	16	7
17	15	3	23	17	4	21	17	7
21	18	3	22	17	4	18	16	7
22	16	3	19	17	5	24	17	7
23	16	3	19	15	5	20	15	7
19	18	3	21	19	5	22	18	7
21	17	4	18	14	5	21	17	7
18	20	4	22	19	5	15	16	8
26	14	4	21	17	6	21	17	8
24	17	4	18	14	6			<u></u>
18	19	4	25	18	6	1	Averages	
19	15	4	23	14	6	20.9	16.7	5

Ratio of new and old segments, 30 p. c.

The number of removed and old segments in this experiment differs immaterially from those of the control (see Table I, A). On the contrary the average number of regenerated segments

shows a considerable reduction, the decrease being on the average almost two segments. The percentage of new segments is only 30, and as compared with that of the control (40.7) shows that under the action of the chloretone there were formed 10 new segments less to every 100 old segments than under normal conditions.

The experiment repeated in 1908 yielded practically the same result, as will be seen from the Table XIII.

TABLE XIII

Chloretone

August 24 to September 4 (1908)

d	CONTROL		LORETONE OI per cent	i i	oretone 5 per cent						
	Number of segments										
Old	Regenerated	Old	Regenerated	Old	Regenerated						
14	4	18	2	18	2						
16	4	19	2	20	2						
13	4	20	2	. 14	3						
19	4	19	2	18	' 3						
16	5	19	2	19	3						
19	5	17	3	21	3						
15	6 ,	17	3	17	4						
19	6 1	20	4	22	4						
		21	5	ı	•						
Av. 16.4	4.8	19	2.8	18.6	3.0						

The number of regenerated segments in the control varies from 4 to 6, while in a 0.001 per cent solution of chloretone it varies from 2 to 5, and from 2 to 4 in a 0.005 per cent solution.

In order to verify the results obtained from the experiments recorded in Table XIII and also to meet the possible criticism that the increase or decrease in the rate of regeneration might not have been caused by the substances in the solutions employed but have been purely accidental, the worms of all the previous experiments were left to continue their regeneration for a few

weeks more. They were then all transferred to normal seawater under conditions as nearly alike as possible, but I was unable to detect any differences in the regenerating capacity of the worms during this period of time, although chloretone, especially the stronger solutions, seems to leave an after effect.

# Second Series of Experiments

These experiments were performed in order to test the effect of atropine and digitalin on worms in the process of regeneration. Stock solutions of 0.001 per cent of atropine sulphate and of digitalin (Merck) were prepared in sea-water, and the solutions used in the experiments were made by diluting these stock solutions.

In all the experiments to be described hereafter the regenerating worms were submerged in the solutions for the full period of their regeneration. The water in the experiments and in the controls was frequently changed. The worms used in these experiments were collected on the same day and from the same locality.

In Table XIV are summarized the data pertaining to the control experiment.

It will be seen from this table that the average number of regenerated segments is 6.1 (4 to 8 segments), and that the ratio of the number of new to that of old segments is 36.3 per cent.

## Experiments with Atropine

The atropine solutions were of two different strengths: 0.001 and 0.0001 per cent. The effect produced by these solutions upon regenerating worms, so far as the rate of regeneration is concerned, is recorded in Tables XV and XVI.

In Table XV the average number of regenerated segments (3 to 6) is 4.7, which presents a decrease as compared with the control. In Table XVI the number of regenerated segments varies from 3 to 8, with an average of 6.4, showing that the atropine in such a dilute concentration is ineffective.

Expressing the effect of the stronger atropine solution upon the rate of regeneration of new segments in terms of percentages, it

will be seen that the 0.001 per cent solution of atropine sulphate (1:100,000) lowered the regenerative capacity 10 per cent (36.3 to 26.5).

TABLE XIV

Control (For experiments recorded in Tables XV, XVI, XVIII and XIX)

August 28 to September 11 (1907)

NUMBER OF SEGMENTS

Old	Regenerated	Old	Regenerated	Old	Regenerated
18	2	17	6	19	7
18	4	17	6	15	7
15	4	17	6	16	7
19	4	20	6	15	7
15	4	15	6	13	8
18	4	16	6	16	8
17	5	18	6	17	8
18	5	14	6	14	8
19	5	16	6	16	. 8
14	5	17	7	16	8
15	5	17	7	17	8
15	5	19	7	17	8
17	5	19	7	19	8
19	5	17	7		
16	5	19	7	Av	rerage
18	5	19	7	16.8	6.1

Ratio of new to old segments, 36.3 p. c.

In the course of the first few days there was hardly any difference between control and experimented worms. The difference, however, became quite noticeable at the end of the first week. This is especially true of the worms treated with 0.001 per cent of atropine, in which the "Anlagen" of parapodia fail to develop for some time, and the segments already formed are poorly differentiated from one another. Even at the end of two weeks the depressing effect of the solution could still be easily observed in the diminished growth of the parapodia and of the regenerated tail as a whole, as compared with those parts in worms regenerating under normal conditions.

TABLE XV
Atropine 1: 100,000
August 29 to September 12 (1907)

NO. OF SEGMENTS								
Old	Regenera ed	Old	Regenerated					
16	3	17	5					
18	3	20	5					
16	4	15	5					
17	4	19	5					
18	4	20	5					
16	4	17	6					
17	4	18	6					
19	4 :	4 18	18 6	4 18	4 18	4 18	4 18	6
17	5 .	19	6					
17	5		- '					
18	5	Av	rerage					
20	5	17.8	4-7					
20	5							
	1		1					

Ratio of new to old segments, 26.5 p.c.

TABLE XVI
Atropine 1: 1,000,000
August 30 to September 13 (1907)

' NO. OF SEGMENTS

Old	Regenerated	Old	Reg	enerate
15	3 1	16	!	7
19	4	17	1	7
14	5	17		7
16	5	18		7
16	5	17	,	8
16	6	18		8
16	6	18	1	8
16	6	20	i	8
17	6 -		1	
16	7	A	verage	:
17	7	16.8	1	6.4
17	7			-

Ratio of new to old segments, 37.8 p.c.

The experiments with atropine were repeated in 1908. The atropine sulphate was then used in three different concentrations of 1,3 and 10 parts to 200,000 parts of sea-water  $(\frac{1}{2000}, \frac{3}{2000})$  and  $\frac{10}{2000}$  per cent). The results are shown in Table XVII.

TABLE XVII

Atropine
August 13 to August 23 (1908)

CON	rrol				ATROPINE I : 200,000		ATROP	
Old Segment	Regener- ated Segments	Old ated Old ated				Old Segments	Regener- ated Segments	
18	3	13	3	. 14	3	14	3	
14	4	17	3	17	3	15	3	
15	4	17	3	18	3	17	3	
15	5	20	4	16	4	18	3	
15	5	14	5	16	4	15	4	
18	5	16	5	16	4	17	4	
19	5	16	5	18	4 1	17	4	
17	6	17	5	17	5	18	4	
		17	5			19	4	
;	1	18	5	,	· -	21	4	
:	;	16	6	,	'			
		17	6	,	'			
•	1	19	6	ı I				

Average numbers of segments

16.4	4.6	16.7	4-7	16.5	3.8	17.1	3.6

It will be seen from these data that the number of regenerated segments in the control (sea-water) and in the weakest concentration of the atropine varies from 3 to 6, but in the strong solution (10:200,000) from 3 to 4. Comparing these data with those of the previous year, it will be found that in concentrations greater than 1:100,000 the atropine produces a retarding effect upon the rate of regeneration, while in weaker concentrations it is ineffective.

## Experiments with Digitalin

Digitalin is highly poisonous and can be used only in exceedingly attenuated solutions. I tested the influence of a 0.0001 per cent (1:1,000,000) and 0.00001 per cent (1:10,000,000) solution of digitalin on the rate of regeneration.

The data from this experiment are contained in Tables XVIII and XIX.

TABLE XVIII

Digitalin 1: 1,000,000

August 29 to September 12 (1907)

NUMBER OF SEGMENTS								
Old	Regenerated							
18	3							
15	4							
19	4							
18	4							
17	4							
17	4							
17	4							
14	5							
16	5							
. 18	6							
Average 16.9	4.3							

Relation of new to old segments in per cent, 25.5

From these tables it may be seen that the worms subjected to the action of the 0.0001 per cent solution of digitalin (1:1,000,000) regenerated on the average 4.3 segments (3 to 6 segments), and that the relation between the new and the old segments is 25.5 per cent. This of course indicates a decrease in the regenerative capacity as compared with the control (Table XIV, p. 623). The weaker solution of digitalin (1:10,000,000) produced no effect upon the regenerating worms, and the regeneration was normal.

TABLE XIX

Digitalin 1: 10,000,000

August 30 to September 13 (1907)

NUMBER OF SEGMENTS							
Old	Regenerated	Old	Regenerate				
13	4	16	7				
16	4	17	7				
17	4	17	7				
18	5	17 7					
20	5	17	7				
16	6	19	7				
16	6	16	8				
17	6	16	8				
18	6	17	8				
16	7	17	8				
17	7	17	8				
18	7 -						
19	7	A٠	verage				
		17	6.5				

The experiments with digitalin were repeated in 1908 and many different strengths were tested. It was found impossible to keep worms alive for any length of time in digitalin even of such a weak concentration as 1:800,000. In Tables XX and XXI are brought together the data of experiments with digitalin both weaker and stronger than the weaker solution employed in 1907.

TABLE XX

AUGUST 21-AUGUST 31 (1908)	-		NUL	(Ber	OF S		ENTS			AVERAGE
Digitalin 1:1,600,000 $\begin{cases} Old \\ New \end{cases}$	15	15	15	15	3	17 3	20	15 4	16 4	16.1 3.2

### TABLE XXI

SEP. 3-8EP. 13	(1908)	NUMBER OF SEGMENTS				
Digitalin 1 : 3,200,000	{ Old New	15 15 16 19 19 21 15 16 16 16 17 18 16 17 18 20 16 17 17 18 18 16 17 19 3 3 3 3 3 4 4 4 4 4 4 4 4 4 4 5 5 5 5 5	17.2			

In Table XX, referring to an experiment with digitalin in a concentration 1:1,600,000, it will be observed that the number of regenerated segments varies from 3 to 4, the former ones predominating. In the control of this experiment the number of regenerated segments varied from 3 to 5, and 4 was the predominant number. So that in this case, as also in the concentration of 1:1,000,000, the rate of regeneration was decreased. In Table XXI are given the results of an experiment with digitalin in a concentration of 1:3,200,000. The number of regenerated segments varied from 3 to 6, while that of its control varied only from 2 to 4. Not only has the number of segments regenerated been greater in this solution of digitalin than in normal seawater, but the regenerated tails themselves were larger.

If we compare all the data from experiments with digitalin, it will be found that solutions of this reagent may exert either a highly toxic effect, killing the animals, or it may exert a retarding or a stimulating effect or none at all, depending upon its concentration. The limit of toxicity lies somewhere between concentrations I:800,000 and I:1,000,000; the lower limit of its effectiveness being somewhere in the neighborhood of I:10,000,000, while in concentrations I:1,000,000-I:2,000,000 it is depressing, and in concentrations I:3,000,000-I:4,000,000 it is stimulating in its action.

# Third Series of Experiments

In this series of experiments was investigated the effect of other alkaloids, such as strychnine and pilocarpine, upon the rate of regeneration. Solutions of 0.001 per cent of either strychnine or pilocarpine proved extremely toxic to the worms, all of which invariably died in these solutions. It may, perhaps, not be amiss to mention here that the worms were not killed by these solutions immediately, but that they continued to live in them for 6 to 7 days. The poisons, however, affected the worms in a curious fashion. In all of the worms the intestines became everted and thrust out through the mouth. None of these worms ever regenerated. Even in the much more diluted solutions worms were occasionally found that were affected in a similar

way, thus showing how very differently each worm reacts to the same medium; but in the 0.001 per cent solution not a single worm escaped the deleterious action of the poisons.

In Table XXII are given the data of an experiment which will serve as the control for this series.

TABLE XXII

Control

(For Experiments Recorded in Tables XXIII-XXVI)

August 31 to September 14 (1907)

Old	Regenerated	Old	Regenerated
14	3	16	6
16	3	16	6
14	4	18	6
17	4	18	6
18	4	15	7
16	. 5	17	7
16	5	17	. 7
18	5		
16	5	A	verage
16	5	16 3	<i>r</i> 2
17	5	.0 3	5.3
15	6		
16	6		

Ratio of new to old segment, 32.2 p. c.

The average number of segments here is 5.3 (from 3 to 7), and the index of regeneration, i. e., the ratio of the number of segments to that of the old ones, is 32.2 per cent.

# Experiments with Strychnine Sulphate

Two solutions of strychnine sulphate (1:1,000,000 and 1:10,000,000) were employed, since the 1:100,000 solution was so highly toxic to the worms. The effects upon the rate of regeneration produced by both of these solutions are quite similar, as will be noticed from Tables XXIII and XXIV.

TABLE XXIII

Strychnine 1: 1,000,000 August 31 to September 14 (1907)

NUMBER OF SEGMENTS									
Old	Regenerated	Old	Regenerated						
16	2	16	5						
17	2	16	5						
15	3	17	5						
17	3	17	5						
17	4	17	6						
17	4	17	6						
18	4	17	6						
18	4								
18	4	Av	erage						
15	5 .	16.7	4-3						
15	5								

Ratio of new to old segment., 26 p. c.

TABLE XXIV

Strychnine I: 10,000,000 August 31 to September 14 (1907)

-	NUMBER OF		-
Old	Regenerated	Old	Regenerated
14	3	15	5
15	3	16	5
16	3	16	5
17	3	17	5
17	3	17	' 5
15	4	17	5
16	4	16	6
17	4	17	6
18	4	17	' 6
17	5 -		
18	5	Av	erage
18	5		,
18	5	16.5	4.5

Ratio of new to old segments., 27.2 p. c.

In Table XXIII are recorded the results of the experiment with the 1:1,000,000 solution of strychnine. The number of new segments varies from 2 to 6, and the average number of regenerated segments as well as the numerical relation of those segments to the old segments are lower than the corresponding numbers in the control.

Table XXIV, which refers to worms treated with 1:10,000,000 solution shows very much the same thing, although the effect is not quite as distinct as in the previous case.

Thus strychnine produced a depressing effect upon the regenerating worms, which resulted in a lowering of the index of regeneration by about 5 to 7 per cent as compared with that of the control.

# Experiments with Pilocarpine Hydrochloride

Solutions of pilocarpine hydrochloride in sea-water were prepared of three different strengths—I: 100,000, I: 1,000,000 and I: 10,000,000. The first of these solutions was too poisonous in its action, and as already stated, caused death to all of the worms.

In Table XXV are given the data of an experiment with the 0.0001 per cent solution of pilocarpine (1:1,000,000) upon the regenerating worms.

TABLE XXV

AUGUST 31-SEPTEMBER 14 (1907)	-	Ì	- NUM	 (BER	OF 6	EGMI	NTS		AVERAGE
Pilocarpine 1 : 1,000,000	Old New	17	18 4	18 4	18 4	19	18 5	18 6	18 4·3

The average number of segments here is 4.3 and comparing these data with those of the control (Table XXII) it will be found that the regenerative power of the worms suffered a reduction.

In Table XXVI are presented the data with regard to the action of the 0.00001 per cent solution of pilocarpine (1:10,000,000) upon the regenerating worms.

TABLE XXVI

Pilocarpine 1: 10,000,000 August 31 to September 14 (1907)

	NUMBER OF	EGMENTS	
Old	Regenerated	Old	Regenerated
15	4	16	6
16	4	18	6
17	4	16	7
16	5	16	7
17	5	17	7
17	5	18	7
18	5	15	8
16	5	17	8
17	5		
18	5	Λ	erage
14	6	AV	crage
15	6	16.5	5.8
16	6	_	

Ratio of new to old segments., 35.4 p. c.

In this case the average number of regenerated segments, likewise the numerical relation between the new and the old segments, are somewhat greater than in the control, but a conclusion as to the stimulating influence of pilocarpine could not be drawn safely.

I, therefore, repeated the experiments with pilocarpine in 1908. A concentration of 1:800,000 was found absolutely poisonous, so that a concentration of 1:1,000,000 is probably not far above the limit of toxicity. Solutions of a concentration of about 1:2,000,000 produce a more or less retarding effect upon the rate of regeneration. Weaker solutions of pilocarpine (1:3,200,000) produce a distinctly stimulating effect upon regenerating worms, as will be seen from Table XXVII.

TABLE XXVII

September 3—September i	3 (1909)	NUMBER OF SEGMENTS	AVERAGE		
Pilocarpine 1:3,200,000	{Old New	15 16 16 17 17 18 17 18 19 19 20 19 16 17 18 19 20 20 21 16 18 3 3 3 3 3 4 4 4 4 4 5 5 5 5 5 5 5 5 5 5			

The list of instances of the stimulating or the inhibiting effect of various reagents on different manifestations of life could be easily lengthened. From all these it may be safe to infer that similarity of the effects produced by reagents on various functions of living organisms—be they growth, development or regeneration—implies a general cause, which is to be found in the augmentation or depression of the protoplasmic activity of the individual cells of these organisms, which is really the only source of energy for all functions.

# VII CONCENTRATION AND DILUTION OF SEA-WATER AS A FACTOR IN THE RATE OF REGENERATION

Loeb ('92) found that upon bringing stems of hydroids into sea-water of various degrees of concentration the number of regenerating hydranths is either increased or decreased depending upon the concentration, the optimum, however, being sea-water diluted to about 66 per cent. Similar results were obtained since then by Snyder, Peebles and some other investigators. This interesting fact, of what is supposed to be the effect of osmotic pressure upon regenerating animals, calls for some more experiments for its final establishment. I therefore devised a number of experiments with the purpose in mind of further studying this phenome-In 1907 my material unfortunately ran short, so that only three experiments were performed. Briefly stated, the experiments and the results were as follows: Two solutions of sea-water were used; in one case the sea-water was diluted with an equal volume of distilled water, in the other with one-third its volume of distilled water, giving respectively a 50 per cent and 75 per cent mixture. The 50 per cent mixture was very injurious to the worms, and though some of them lived for quite a long time, none had proliferated any new tissue. The 75 per cent mixture acted less injuriously, but even it produced a marked retarding influence upon the rate of regeneration. The concentration of the sea-water was brought about by the addition of 2 per cent of MgCl, to the sea-water. In this case also the animals suffered a considerable reduction in the power of regeneration.

This subject was taken up again in my work during the summer of 1908. Upon diluting sea-water to 75 per cent of its original strength I obtained invariably a distinct retardation of the rate of regeneration. The effect made itself manifest not only in the smaller number of regenerating segments, but likewise in a diminution in length of the regenerated tails. It will be seen from the Table XXVIII that the number of regenerated segments varied from 2 to 5, two and three segments being the most common numbers.

TABLE XXVIII

SEPTEMBER 5 TO SEPTEMBER	15 ('08)	NUMBER OF BEGMENT	8
Sea-water-75 parts. Dist. water-25. parts.	∫ Old New	18 18 19 15 16 17 19 16 17 18 1 2 2 2 3 3 3 3 3 3 3 3 3	8 16 16 17 17 17 18
	(	[ ]	,   +   +   +   3   3

It should be mentioned, however, that of all the experiments with sea-water diluted to 75 per cent this shows less of the deleterious influence upon the formation of new segments than any other; it is used here because it belongs to the series of experiments the data of which are taken as a basis for this chapter.

In the control experiment (Table XXIX) the number of regenerated segments varied from 3 to 5, but, as has been said above, the tails were much larger.

TABLE XXIX

SEPTEMBER 5 TO SEPTEMBER 15 (1908)			NUMI	BER (	DF 81	GME	NTS			
Control (Sea-water)	Old New	15	15	16 3	18 3	17 4	17	20	16 5	19 5

In sea-water of strength greater than 75 per cent, as for instance sea-water diluted to 80, 85, 90 or 95 per cent its original strength there was exerted upon the regenerating worms neither a retarding nor an accelerating influence. As will be seen from Tables XXX and XXXI, there is practically no difference between the worms of the control and the experiment so far as the rate of regeneration is concerned.

TABLE XXX

SEPTEMBER 5 TO SEPTEMBER 15 (1908)					NUMBER OF SEGMENTS									
Sea-water—80 parts Dist. water—20 parts	{ Old New	16 18 3   3	19 14 3 4	17 1	19 16 1	18 14 1 4	17 1	18 4	19	6 17 5 5	19 5	20 5		

TABLE XXXI

SEPTEMBER 5 TO SEPTEMBER		-		NU	MBER	 OF	8 E GN	ENTS	3		-	-	•	
Sea-water—90 parts Dist. water—10 parts	{ Old New	16 3	17	19	17	19	18 4	18 4	16 5	18 5	<b>20</b>	16 5	18	

The same results were also obtained from other experiments. In these, too, there was no good evidence of a beneficial or a detrimental influence.

The concentration of sea-water above the normal was accomplished by adding various quantities of a gram-molecular solution of MgCl<sub>2</sub> and KCl. As regards the last substance, it may be stated that, even in quite small amounts, it proved absolutely fatal to the animals, and they died very rapidly. Of the molecular solution of MgCl<sub>2</sub> various quantities, from 1.25 cc., to 15 cc. were used, and to these enough sea-water was added to make 100 cc.

When as many as 15 cc. of this solution are added to the seawater (85 cc.), there is no trace of regeneration, even at the end of ten days after the operation, the worms dying out in great numbers. In a mixture containing 10 cc. of the solution a few of the worms regenerated even as many as three segments, while other worms did not regenerate at all or died. The use of 5 cc. of the MgCl<sub>2</sub> solution does not in any way hinder the normal process of regeneration, even as many as 5 segments being regenerated. Although there is no advantage of which one could be certain in favor of worms regenerating in sea-water of this concentration, there is also no apparent disadvantage. When, however, smaller quantities of MgCl<sub>2</sub> are employed, there frequently occurs an acceleration of the rate of regeneration, which will be more or less pronounced in different experiments. In Tables

XXXII and XXXIII are given the data of experiments in which were tested solutions containing 95 cc. of sea-water + 5 cc., and 90 cc. of sea-water + 10cc. of a half-molecular solution of MgCl<sub>2</sub>.

TABLE XXXII

SEPTEMBER 5		NUMBER OF SEGMENTS									
Mg Cl₂ 5 cc. + 95 cc. Sea-w.	Old New	14 18 18 19 20 12 15 15 16 16 18 19 14 14 16 17 20 14 16 4 4 4 4 4 4 4 4 4 5 5 6 6 6	18								

TABLE XXXIII

SEPTEMBER 5 TO SEPTEMBER 15 (1908)				NUMBER OF SEGMENTS											
Mg Cl <sub>2</sub> 10 cc. + 90 cc. Sea-w.	Old 17 New 3	18	19	14	16 4	17	18	17	18	19	14	16 5	17 5	19	14

The beneficial influence which the sea-water slightly concentrated by MgCl<sub>2</sub> exerted upon the process of regeneration is quite obvious not merely from the greater number of regenerated segments (Table XXXII) but also from the fact that very few worms, as compared with the corresponding control experiment, failed to regenerate in sea-water of this concentration.

Loeb in summing up his interesting chapter—"Ueber die Abhängigkeit der Regeneration bei Tubularia von der Konzentration des See-wassers"—says: "dass die Wasser-aufnahme eine wesentliche Bedingung der Regeneration bei Tubularia (und wahrscheinlich bei allen Thieren) ist." On the basis of my experiments, it would seem scarcely probable that this generalization could be applied to Podarke. On the contrary, the addition of an appropriate quantity of MgCl<sub>2</sub> to the sea-water may accelerate the regenerative process in this animal, while dilution of the sea-water may either leave the worms unaffected, or else it may impair their regenerative capacity.

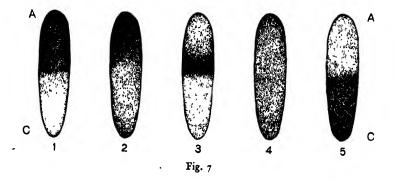
It would, therefore, be legitimate to question whether the accereration observed in Tubularia is due primarily to osmotic pressure. Perhaps there is some other factor responsible for the acceleration. In fact, Miss Peebles ('08), in a recent paper on grafting experiments with Tubularia, pointed out that her experiments on the influence of concentration of the sea-water yielded results which indicate "that the great increase in size of the hydranths and the rapidity of their formation in dilute sea-water is due to something more than the difference in osmotic pressure." She believes that there are some organic substances in the seawater which exert a retarding effect upon regenerating stems of Tubularia, and that this effect may be abolished by diluting the sea-water, so that "the growth which we consider so unusual is really no more than the normal rate under optimum conditions."

This suggestion is in a sense in accordance with the results of my experiments, where, as we have seen, KCl and MgCl<sub>2</sub> produced opposite effects upon regenerating worms in virtue of their different chemical properties, since the osmotic pressure was the same in both cases. It is likely, therefore, that MgCl<sub>2</sub> produces a favorable effect on the rate of regeneration when present in the optimum quantity, as do other substances employed in the preceding experiments, because of its special properties and not through a change in osmotic pressure.

### VIII COMPENSATION

It was remarked in the beginning of this paper that the worms have a chitinoid covering, usually of a seal-brown color. When a new tail begins to grow out this covering is at first lacking and the tail is therefore more or less translucent. The new tail soon acquires this chitinoid covering, but while the covering is being formed here, it gradually disappears from the old piece. In Fig. 7 an attempt is made to represent this process diagrammatically.

In this figure (1) is supposed to represent the condition when the old piece is thickly covered over, and the new tissue is still "naked." From this time on, the covering on the old part changes from dark brown to light shades of yellow. The change may first take place either on the front portion of the animal (3) or on the portion nearest to the regenerating tissue (2) or occasionally on the sides of the old part of the animal. While this change goes on, the new tissue becomes coated with a brown layer, which gets gradually thicker and also darker. The process continues until the color of the cuticula is uniform on both sides of the cut surface, so that it becomes difficult, at times even impossible, to distinguish the old from the regenerated tissue, as shown at (4) on the figure. The process does not always end here, however. The curious thing sometimes happens that instead of remaining in this condition of apparent equilibrium, the chitinoid covering continues to thicken above the regenerated part and to disappear from the old part, so that finally a stage is reached, like that shown in (5) of the Fig. 7, which is just the



reverse of the condition found in (1), i. e., the regenerated tissue is now covered by a seal-brown layer and the old part has become translucent.

In those few exceptional cases where the worms had no covering at all, the new tails also remained "naked." The same is true of worms treated with a 0.0001 per cent solution of strychnine, in which case the covering disappeared entirely, and none was formed over the regenerated tail.

This phenomenon, it seems to me, furnishes additional evidence in favor of the view that it is the regenerating organism as a whole which is engaged in the process of building up the lost parts.

## IX SUMMARY

- I The rate of regeneration varies with the "level" of the worm's body at which the cut has been made, being greatest when the cut is in the anterior region and decreasing gradually for cuts in more posterior regions.
- 2 In the process of posterior regeneration there are certain definite stages whatever the level of the cut: there is invariably a lapse of some time, which varies with different individuals and under different conditions, before new tissue is proliferated; this is followed suddenly by a period of rapid formation of new segments, to be in turn followed soon by a period of slower regeneration. From this time on the rate of regeneration is constantly decreasing until finally the process is brought to a standstill.
- 3 The difference in the rates of regeneration from different levels appears at the very start of the regenerative process, the period of time during which there is as yet no regeneration taking place being longer in the case of worms cut near the posterior end than those cut nearer the anterior end; moreover, the number of segments regenerating during any given period remains smaller in the former case than in the latter throughout the entire process of regeneration. The difference in the rates of regeneration from different levels is, thus, a continuous difference, not being manifested at any particular stage in the process.
- 4 The period of very rapid formation of new segments is coincident with a period of very slow growth of the existing regenerated segments, while the period of slow formation of segments is accompanied by a vigorous growth of those already differentiated. The formation of new segments and their subsequent growth seem therefore to be reciprocal processes.
- 5 After a second operation Podarke regenerates at a considerably slower rate than after the first operation. This slowing down of the regenerative process after the second operation is especially well marked during the first few days after the operation, as compared with the rate of regeneration for a similar period of time after the first operation. After a certain time, however, the worm may again resume the normal rate of regeneration.

- 6 An abundant supply of food is favorable for the regenerating worms in this sense that fed worms regenerate both more segments and longer tails than worms that are not fed. But it is important to notice that in fed and in starved worms alike the rate of regeneration varies with the level from which they regenerate, in accordance with the rule given in the first clause of the summary. It follows from this circumstance, that food must be eliminated as a factor in accounting for the difference in the rates of regeneration at different levels.
- 7 The rate of regeneration can be artificially modified by subjecting worms to the influence of various organic substances, which produce either a stimulating or a depressing effect upon their protoplasmic substance.
- 8 Alcohol may either increase or decrease the rate of regeneration depending upon the strength of the solution, whereas chloretone decreases the power of regeneration.
- 9 Atropine sulphate in certain concentrations retards regeneration.
- 10 Digitalin may either stimulate or retard the rate of regeneration, the effect being entirely dependent upon the strength of the concentration. In concentrations slightly weaker than the probable limit of toxicity (1:800,000) this alkaloid produces a retardation, whereas in a still weaker concentration it acts stimulatingly on the regenerative process; by further diluting the solution it will be rendered quite ineffective.
- 11 Strychnine sulphate invariably produces a retarding influence upon regeneration.
- 12 Pilocarpine hydrochloride either stimulates or diminishes the power of regeneration, depending upon the concentration. Solutions only slightly weaker than 1:800,000 (the probable limit of toxicity) produce a retarding effect, but with greater dilution (1:3,200,000), a distinct stimulating effect is produced. By still further dilution this substance (like digitalin), is made entirely ineffective.
- 13 Sea-water diluted to 95 per cent or even to 80 per cent of its normal strength produces no apparent influence upon the regenerating worms, the rate of regeneration remaining practically

the same as in normal sea-water. Sea-water diluted to 75 per cent its original strength produces a retarding effect on the power of regeneration, and a dilution to 50 per cent is very injurious to the worms.

14 The addition of MgCl<sub>2</sub> to the sea-water may produce a favorable or an unfavorable effect on the power of regeneration according to the amount employed. More than 5 cc. of a molecular solution may retard or even completely inhibit the regenerative process, whereas smaller quantities (5 cc. of ½ solution) may often be very beneficial both to the regeneration and growth of new segments. It is probable that the beneficial effect on the rate of regeneration produced by the addition of a small amount of MgCl<sub>2</sub> to the sea-water is not the outcome of a change in osmotic pressure alone.

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## THE INFLUENCE OF THE NERVOUS SYSTEM IN REGENERATION<sup>1</sup>

BY

#### A. J. GOLDFARB

(WITH TWENTY-THREE FIGURES)

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<sup>1</sup> I wish to express my indebtedness to Prof. T. H. Morgan for much stimulating criticism, to Prof. E. B. Wilson, for permission to use the Columbia Table at the Marine Biological Laboratory, Woods Hole, Mass., and to the Hon. Wm. Bowers, Commissioner of Fisheries, for a brief stay at the laboratory at Woods Hole.

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#### INTRODUCTION

In the more highly organized animals, the nervous system is the dominant influence that largely coordinates the activities of the different parts of the organism. Within recent years a number of important functions formerly attributed to it have been removed from its sphere, and in some instances at least have been definitely assigned to other agencies. The influence of "trophic" stimuli has been seriously questioned. The activity of certain glands formerly believed to be regulated by nervous influences has been shown by Starling to be controlled by chemical agencies. has also been held that nerves provided the morphogenetic stimulus to development, differentiation and regeneration of embryos. But the investigations of Schaper, Rubin, Goldstein, Harrison, Born, Roux and others clearly demonstrated that these phenomena take place in the absence of the nervous system. Development and regeneration take place in plants, in protozoa, and in eggs of various kinds, yet no one speaks of the necessity for assuming the presence of a nervous system in these cases. In adult animals however, especially among the higher groups, regeneration is believed to depend directly or indirectly upon the central nervous system. This belief gained ground not so much as a direct result of experimentation, but rather by a process of elimination of other factors. Even those (Goldstein, Wolff) who are convinced that during the larval stages regeneration is independent of nervous influences are just as convinced of its supreme importance in the adult.

Herbst has taken the extreme position that the sensory ganglia of the nervous system exert this stimulus. Herbst, as is well known, found that after extirpating the whole of a decapod eye, an antenna-like organ was regenerated. His results were corroborated by Przibram, Zeleny and Morgan. Recently Steele extended these experiments and found that the removal of the whole eye and stalk in hermit crabs resulted in the formation of a heteromorphic structure, not unlike that obtained by Herbst. About 30 per cent of the animals produced such structures. In Crangon however very few were produced, and in Palæomonetes, heteromorphosis could not be induced at all. She corrob-

orated Herbst's observation that the level of the injury determined the kind of regeneration; for, the removal of the entire eye, stalk included, resulted in an heteromorphic structure; when the distal end, including about one-third of the ganglia, was cut out, an eye regenerated. When, however, Steele amputated intermediate between these levels, no regeneration whatsoever occurred. If the presence of the sensory ganglia, in whole or in part, conditioned the regeneration of the decapod eye, it is difficult to understand why they should not exercise their power in other species of decapods, and in certain mollusca (Carrière); and if the ganglia are necessary in the regeneration of the eye, why are they not equally important in the regeneration of the antennæ of the same animal?

Our evidence concerning other adult animals will be found in each of the following sections.

Of special interest are Wolff's experiments wherein he sought to ascertain "whether the hind legs of a Triton, whose nervous connections with the central nervous system had been severed, would regenerate in the same manner as in an uninjured animal."

He removed the nerve cord from the plexus cruralis of a number of the animals of which but three survived. These regenerated the missing leg. He then sought to remove both motor and sensory cells from the plexus. Only six animals survived. These also regenerated, but the leg contained a reduced number of toes. Of these six individuals, he tells us, four displayed some degree of movement within three months after the operation, i. e., there was a return of sensory and motor functions of the limbs. Nevertheless the malformation persisted. "Die oben erwähnten sechs Ausnahmen (haben) mit der denkbar grössten Bestimmheit einen Einfluss des Central-Nervensystems auf den regenerativen Vorgang nachgewiesen." He also drew the conclusion that "Diese Fälle lieferten einen Beweis von der Einwirkung des Nervensystems auf morphogenetische Vorgänge." His final position seems to be that during the early stages in regeneration no nervous stimuli are needed, but that later, unless they be present, atypical structures are produced.

Barfurth '01; Wintrebert '03; Godlewsky '04; Hines '05 also made some experiments on adult animals, with the same general

problem in mind. Their conclusion, though based on very incomplete data, as will be later shown, is however correct.

It is the purpose of this paper to bring together a number of experiments bearing on the relation of the nervous system in the regeneration of adult animals, to find out whether it exerts any direct or indirect influence upon the regenerated organ or upon the process of regeneration, and to determine whether its absence is any more potent a factor in disturbing the normal developmental processes than any one other tissue, such as muscle, bone, etc. The following animals were studied: (1) Diemyctylus viridescens (newt), (2) Frog tadpoles (advanced stage), (3) Lumbricus, (4) Asterias vulgaris, (5) Dendrocœlum lacteum.

## REGENERATION IN THE NEWT (DIEMYCTYLUS VIRIDESCENS)

Newts and salamanders of various kinds have furnished the most common material for experiments intended to show the relation of the central nervous system to morphogenesis. Unfortunately these experiments, with possibly one or two exceptions, fail to make clear the exact nature and extent of the injury to the nervous system. We do not know whether some or all of the nerve cells or fibers supplying the amputated region were destroyed, or whether subsequent changes may not have permitted innervation of this region. Any conclusions based upon such insufficient data are of doubtful character.

Diemyctylus shows a remarkably well-developed power of regeneration. Legs, tail and lens of the eye are replaced readily. In these studies the regeneration of the limbs and tail only will be dealt with, and, for the sake of clearness, will be treated separately.

# Regeneration of the Leg after Different Injuries to its Nerve Supply

The hind legs are supplied by three pairs of nerves that take their origin in the lumbo-sacral plexus, Fig. 1. The anterior two pairs are considerably thicker than any of the adjoining spinal nerves, and can therefore be readily distinguished from them. These nerves will be referred to as plexus nerves I, II and III, respectively. They branch to form the obturatorius and cruralis on the one hand, and the ischiadicus on the other. The last again divides into the peroneus and tibialis, etc., of the leg.

Various attempts were made to sever the nerve connections of the hind legs. The difficulty was in making certain that all nerve impulses to the amputated end of the limb were removed either permanently or for a considerable time at least. For it is well known that the cut ends of nerves may reunite, or secondary connections may be made.

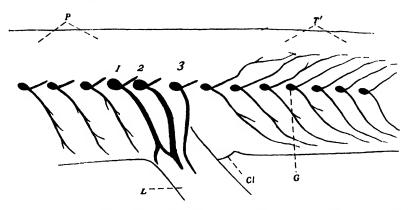


Fig. 1 Distribution of the nerves to the hind leg and tail of Diemyctylus viridescens. Partly schematized. P, pelvic region of body; T', tail region. Innervation same throughout the tail. L, leg Cl, cloaca. 1, 2, 3, first, second and third nerves of lumbo-sacral plexus. G, sensory ganglia.

The simplest operation consists in cutting one or more of the large nerves of the leg. This method is totally inadequate because (1) union of the cut ends might possibly take place by "first intention," i. e., immediately after the operation; (2) connections between the central and peripheral nerves or their branches may be established; (3) the central nerves may grow, independent of the old path, into the peripheral region.

When a piece of the nerve is excised, immediate union of the two ends is prevented, yet this method of operating is open to the first and second objections mentioned above. A third method, and the only one worth serious consideration, consists in destroying the nerve cells. This method is free from the first and second objections and, as will be later seen, from the third also. Its value depends on the certainty that all the nerve cells supplying the leg as well as the nerve cells for a considerable distance in adjacent regions are removed, and on the fact that no regeneration of new cells occurs during the period required for the formation of the limb.

The animals were operated on as follows: The tail was first amputated near its base. Then a fine needle or glass capillary or most often a dentist's broach, technically known as a "pulp-canal cleanser," was introduced into the vertebral canal to any desired depth, to and beyond the lumbo-sacral plexus. The contents, including the spinal cord, its membranes, the roots, etc., were destroyed by repeated movements of the instrument, and the débris withdrawn. One or both hind legs were then amputated. In this way the motor cells supplying the tail and the hind legs are effectually removed. This is evidenced by the complete paralysis of the legs, which after successful operations remained so for over nine months. Even the strongest stimuli failed to produce any reaction. The muscles of the leg lose their tonus, and become considerably shrunken. The muscles of the bladder were likewise paralyzed. The animals ate well and were otherwise in apparently good condition.

About one month after the operation, the time varying with the season of the year, the new leg became visible in both normal and paralyzed individuals. The regeneration in the latter was somewhat retarded. Any severe injury, as will later be shown, whether involving the nerves or any other tissue, retards regeneration. This is partly proven by the following: In control animals the regenerated fore leg appears usually a few days before the regenerated hind leg, though both had been amputated at the same time and at the same level. This slight lead is usually maintained throughout succeeding differentiations. When the hind leg developed first, the lead was sooner or later lost; the fore leg catches up or advances beyong the other. In paralyzed animals a longer period usually intervenes before the appearance of the new fore and hind legs. Once begun, however, the relation of the paralyzed hind and the non-paralyzed fore leg is exactly like that in the control animals.

There often appeared to be considerable differences between the regenerated legs of operated and of control animals. These however were found to be largely due to the indirect consequences following the operation. For example, the paralyzed limb amputated at or below the knee assumes such a position, that the weight of the body largely rests upon the cut surface, thus forcing the regenerating end to grow at a sharp angle to the axis of the stump. The new foot and leg become permanently twisted, which gives the whole limb a very abnormal appearance. The abnormality was further augmented by the degenerating musculature of the stump. Not infrequently the number of toes were reduced from five to four or even three, but this reduction is not uncommon among newts that are injured in other ways, and not rare among control animals.

A variation of the above experiment consisted in first amputating the limbs and at varying intervals thereafter in destroying the lumbosacral plexus. This experiment was intended to test whether the removal of nerve impulses had a retarding or inhibiting or other deleterious influence upon a limb that had already begun to regenerate. No such influence was observed. The rate and extent of growth, the shape and the size of the newly formed limb were virtually the same as in the control series. Once begun regeneration was not affected by the destruction of the motor nerve cells supplying the limb.

In the first series of experiments it is possible that the brief initial stimulus between amputation and paralysis may have sufficed to start the cycle of changes that end in the formation of a new limb or part thereof. That an incredibly short stimulus may suffice to start and maintain the regenerative processes to the completion of an organ was shown by the writer in Eudendrium ramosum.<sup>2</sup> To avoid the briefest initiatory stimulus, the limbs in a third series were amputated after paralysis. The intervals varied from a few days to several months, which in the latter cases at least was sufficiently long for all the motor nerves of the paralyzed limbs to have degenerated. Nevertheless new legs developed.

<sup>&</sup>lt;sup>2</sup> Goldfarb, A. J.: Light as a Factor in the Regeneration of Eudendrium ramosum. Journ. Exp. Zool., 1905.

If it can be shown in these three series (1) that all nerve cells of the cord supplying the hind limb were destroyed, (2) that other motor nerves from regions in front have not grown or connected with those in the paralyzed limbs, (3) that the nerve cord itself has not grown into the lumbar region to supply the limbs with a newset of nerves, it follows that in the adult Diemyctylus viridescens the missing parts of the limb may be restored without the aid or stimulus of motor nerve impulses. In the above three series, whether the limbs were completely or only incompletely paralyzed, or whether paralysis occurred before or after amputation, new limbs regenerated in nearly every instance.

## Lumbo-sacral Plexus Destroyed-Limbs Paralyzed Completely

Many of the operated animals were studied in serial section. The tail, both fore and hind limbs, and the lumbar and thoracic regions of the body were preserved in Hermann's platinic chloride, containing 4 per cent osmic. In some instances corrosive acetic was used. Apathy's silver nitrate and Bielschowsky's silver methods were tried with very uncertain results. As is well known the osmic content of the Hermann mixture stains the myelin of medullated nerve fibers intensely black. The myelin stains deeply even after it has broken down in the familiar Wallerian degeneration and until all the fatty globules thus produced have disintegrated or been absorbed completely, so that the progress of the degenerating and regenerating nerve fibers are readily studied. Surrounding tissues are likewise stained a pale yellow-brown, sufficiently intense in most cases for general use. Sometimes Delafield's Hæmatoxylin was used to emphasize certain structures. in general Hermann's fluid gives excellent results both as a fixing and a staining solution, it is not always to be depended upon, especially when large objects are used. The platinic chloride may penetrate the inner regions more rapidly than the osmic, preventing the fatty tissue from taking the characteristic stain.3

The following animals include those from which at least the

<sup>\*</sup> For this explanation I am indebted to Dr. O. S. Strong.

cord in the lumbo-sacral plexus had been completely destroyed, as determined from a study of serial sections.

No. 1.27. Tailwas amputated 5 mm. from its base. Nerve cord was removed by broach for 20 mm. After this both hind legs were amputated below the knee. Animal preserved fifty-two days after the operation. The hind limbs had regenerated one and one-

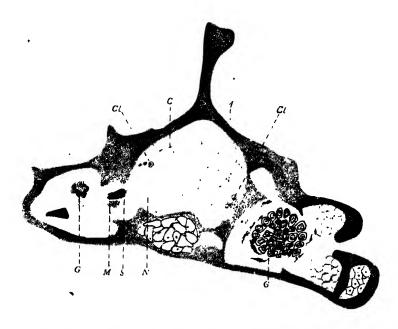


Fig. 2 Cross-section of the spinal column of D. viridescens through the first plexus nerve and ganglia. Shows complete removal of the nerve cord. Inner end of roots shown on the left. A, neural arch; C, connective tissue replacing nerve cord; G, sensory ganglion, end section on the left, median section on the right; Cl, proliferated cartilage; M, inner end of motor root; S, inner end of sensory root; N, space formerly occupied by nerve cord.

half and I mm., respectively, and three toes were distinctly formed on each leg. No movement of the limbs occurred even after strong stimulation.

Sections showed the total absence of the nerve cord, its membranes and the proximal part of the roots from the region in which all three plexus nerves take their origin. The neural canal is partly filled with delicate connective-tissue cells. Fig. 2 is a cross-section not

quite at right angles to the axis of the animal, and through the origin of the first plexus nerves. This particular section was chosen because it shows among other things the proximal end of the roots, and it is seen that they do not extend within the vertebral canal. These roots lead into the spinal ganglion, only a part of which is seen at this level. On the opposite side, the section passes through a more median plane of the ganglion. It will be observed that the ventral or motor root is far more degenerate than its dorsal mate, and that both roots neither connect with the cord nor with the roots on the opposite side. They end abruptly at the neural arch.

The conditions within the neural tube, seen in Fig. 2, persist unchanged throughout the plexus region as well as throughout the tail. There is no trace of a nerve cord or nerve cells.

For the sake of ensuring better fixation, the limbs were frequently preserved separately. In this case it was found on examination that between the spinal column and close to the amputated end of the limb, the nerve fibers were perfectly normal. Near the latter end, however, the myelin had broken down into globules, characteristic of degenerating nerves. No trace of nerve fibers were found in the newly regenerated part of the leg.

Unfortunately, the region anterior to the first plexus ganglion was not preserved, so that no definite statement concerning it can be made. Nevertheless it is quite certain that every source of motor stimulation emanating from the plexus proper, to the very end of the tail, was completely removed and that no regeneration of the cord into this region occurred. The hind limbs were completely and permanently paralyzed, and unable to respond to very strong stimuli. Examination showed that the regenerated structures contained no motor nerves. The nerves like those in the frog degenerate very slowly, for after fifty-two days only that part of the nerves nearest the amputated end had degenerated. The remainder of the fibers appeared quite normal.

According to Waller, Marinesco, Halliburton, Harrison and other investigators nerve fibers may be regenerated and consequently stimuli may be transmitted only when the nerve cells are present. In this case, however, these have been destroyed.

There remains then the possibility that nerves from more cephalad regions might have innervated the limbs. This seems altogether improbable for the following reasons. While we know nothing about the region anterior to the plexus in this case, in other animals to be mentioned shortly, the nerve cord was completely removed from one to five vertebræ anterior to the plexus, a distance sufficiently great to prevent altogether, or at least for a time, the growth of anterior fibers into the leg. Secondly, the greater portion of the nerve fibers do not degenerate till long after the new limb has appeared, i. e., the nerves of the leg exert no chemotactic influence upon the nerves in the more anterior regions. Thirdly, it will also be pointed out later that the presence of a very minute portion of the nerve cord in the plexus region gives some power of movement to the limbs. The total removal from the plexus and adjoining regions is always followed by complete and permanent paralysis of both limbs, so that the latter condition may be taken to indicate the total absence of motor nerve innervation to the rear limbs. These facts may be interpreted in but one way, that the legs regenerated in the total absence of motor innervation.

A brief account of some other cases will make clear several doubtful points and strengthen the conclusion already drawn.

No. 1.34. Preserved fifty days after the operation. The regenerated limb was 2 mm. long, with four toes. No trace of the nerve cord was found beginning about 2 mm. anterior to the origin of the first plexus nerve and as far backwards as the amputated end of the tail. Other features are essentially the same as in No. 1.27 except that degeneration of the nerve fibers had not proceeded as far.

No. 5.3. This animal was also preserved fifty days after paralysis. It differed from No. 1.34 in that the limbs were first amputated, and twenty-five days later the nerve cord was removed. The spinal column was cordless beginning about 2 mm. anterior to the plexus to the end of the tail inclusive. The legs however showed some important changes. Though seventy-five days had elapsed after its amputation, only 1½ mm. had regenerated. Fig. 3 is a longitudinal median section of the nerve between the knee and the amputated end. It is a typical picture of Wallerian degeneration, in a rather advanced stage. Near the amputated end,

the nerve has completely disintegrated. Proximally the globules of myelin are large and close together, distally they decrease in size and number and ultimately disappear completely near the amputated end. In other words, the nerve fibers degenerate proximally. Above the knee signs of degeneration become more and more faint, so that near the base of the leg and within the body as far as the spinal column there is no degeneration whatsoever. This account holds true for all the plexus nerves. It was further observed that degeneration is unequal; fibers in all stages of degeneration are found side by side. This, I believe, is due to a difference in the rate of degeneration of different fibers, the motor ones breaking down far more rapidly than the sensory ones. This difference is in all probability the result of destruction of the nerve cell of the one fiber and not of the other.

The following three individuals were preserved sixty-five days after paralysis. In each, the nerve cord on examination was entirely gone from the lumbo-sacral plexus region and tail. The nerves of the hind limbs were substantially in the same condition as No. 5.3.

No. 1.32 and 1.33. Legs amputated twenty-five days after paralysis. Preserved forty days later. No regeneration.

No. 1.38. Amputated eleven days after paralysis. Parts preserved fifty-four days later. The regenerated leg was 3 mm. long with 4 toes. This was as much as the fore leg had regenerated in the same time.

No. 1.58. Hind legs amputated twenty-three days after paralysis. The animal was preserved eighty-seven days later. The right leg had grown 1½ mm., and showed no external differentiation of toes; the left grew 2 mm., with 4 toes. Sections of the right leg revealed the presence of the elements of the new leg, foot and toes, though they had not been visible externally. Of especial interest are the changes in the nerves. These have completely disintegrated near the amputated end. Near the base, typical degeneration of the fibers (Fig. 3) extends not only to the base of the leg, but within the body about one-fourth the distance towards the spinal column. For some distance centrally, the fibers are quite normal. But closer to the spinal ganglia, some fibers have

begun to degenerate and the degeneration increases centrally, so that next to the ganglia many fibers have disintegrated completely. Others, though separated by spaces, are quite normal. These facts mean that the nerve fibers have degenerated from two separate centers, from the amputated end of the leg proximally, and from the spinal column centrifugally. In this particular animal the two degenerating processes have come closer together than in any of the preceding instances. It should be borne in mind that while



Fig. 3 Longitudinal section of large nerve in hind leg, showing typical Wallerian degeneration Upper part close to the knee, the lower, near amputated level.

centripetal degeneration includes all fibers of the nerve bundle, degeneration in the opposite direction affects the motor fibers only. The nerve cord was absent in the tail and plexus region and for 2 mm. anterior to the plexus.

No. 1.30. Amputated eleven days after paralysis. Preserved 101 days later. Legs had in the meantime grown 4 mm. long and differentiated 4 toes. Conditions were like those in No. 1.58. W. No. 1.39. Right hind leg amputated eleven days after paralysis.

Left hind leg amputated eighty-nine days after paralysis. Animal preserved forty-four days later. There is no trace of the nerve cord not only in the tail and plexus, but for 5\frac{1}{3} mm. anterior to the later. Three pairs of spinal ganglia, cephalad to the first plexus nerve, are not connected with the nerve cord. The condition of the leg nerves is interesting because the motor nerve fibers have degenerated along their entire length from the cord to the amputated end. Near the latter level they have disintegrated completely. The sensory fibers however have degenerated but a short distance from the amputated level. This is in accord with the condition described in No. 1.58. The left leg was amputated eighty-nine days after paralysis, a period sufficiently long to permit extensive degeneration of the nerve fibers. The animal was preserved fortyfour days thereafter, yet in this short interval the element of the new leg had been laid down. There is one striking difference that distinguishes the nerve fibers of this limb from those of No. 1.27, in which the leg had been paralyzed and amputated at the same time and preserved fifty-two days later, viz: the more rapid and profound breaking down of the fibers in the former instance. A similar difference is found in the two sides of 1.39. The nerves in the left leg are only slightly less degenerate than those in the right, though the former had been amputated seventy-eight days after the latter. These facts seem to indicate that when a leg was amputated a long period after the cord had been removed, its fibers degenerated much more rapidly than when the leg was cut off immediately after paralysis.

No. 1.36. December, 16, 1907. Nerve cord removed from tail and plexus and further forward. Right hind leg amputated below knee.

April 4, 1908. Right hind leg preserved, i. e., 110 days after operation. Left hind leg amputated for the first time.

June 6, 1908. Left hind leg preserved, 173 days after paralysis and 63 days after amputation. Examination showed that the cord was absent in the tail, the plexus and farther forward; the plexus nerves were degenerate throughout their lengths. The right leg—second amputation—had grown 5 mm. long, contained 5 well-formed toes, and was indistinguishable from the leg of control

animals. The left leg—second amputation—had been amputated after the total degeneration of the motor nerves, yet it too had regenerated 5 mm. long with 5 normal toes. The nerves in this leg differed from those on the right side only in the less extensive disintegration near the amputated end, due to the difference in time since the amputation of the two sides. There is no sign of medullated motor fibers in either of the regenerated limbs.

No. 1.43. December 16, 1907. Nerve cord destroyed in and beyond the lumbar region.

January 11, 1908. Right leg amputated below knee.

July 14, 1908. Right leg preseved 185 days after amputation, 211 days after paralysis.

March 14, 1908. Left leg amputated.

May 3, 1908. Left leg below knee preserved.

July 14, 1908. Left leg preserved 139 days after amputation, 211 days after paralysis.

The spinal cord was totally absent from the plexus and from three of the vertebræ anterior to the plexus, i. e., for about 5 mm. This animal is particularly worth mentioning because we have indubitable evidence that the sensory nerve fibers have begun to grow back again into the limbs. The nerves give the impression of early stages of degeneration already described. Beginning at the spinal ganglia, the plexus nerves contain normal medullated fibers. The nerve bundles to be sure are very thin, due to the absence of motor fibers. This pseudo-normal condition, which extends but for a short distance towards the base of the legs, is gradually replaced by an atypic region containing many of the degenerate characters already described. Further examination made it clear that this atypical region is a region of active regenerative changes. In the first place the spaces between the fibers increase; secondly, the fibers diminish considerably in diameter; thirdly, these narrow into mere strands of protoplasm with an elongate denser central body; fourthly, the strands disappear leaving lines of spindles which in turn lead distally to the globules of the typical Wallerian degeneration.

No. 1.42. Left leg amputated 110 days after paralysis. Preserved 145 days later or 255 days after paralysis. Regener-

ated 5½ mm. Right leg amputated 89 days after paralysis; 166 days later or 255 days after paralysis, preserved. Regenerated 6 mm.

This animal differs from the preceding one in that the sensory nerves have regenerated still further, in fact to and beyond the amputated level of the leg. Near the cord the plexus nerves stain intensely black, almost as in control animals. It is hardly necessary to add that the regenerated legs contain no motor nerves.

## Lumbo-sacral Plexus Partially Destroyed

The preceding experiments dealt exclusively with those individuals in which not the slightest movement of the hind limbs took place. It was found on examination that in every instance the cord had been totally removed from at least the lumbo-sacral region and usually from the region anterior to it, as well.

The present section considers such animals from which only part of the nerve cord in the region of the plexus had been destroyed, thus allowing a more or less complete recovery from paralysis. The time elapsing between the operation and the first voluntary movement or reaction to stimuli, varied considerably; so also did the extent of recovery vary. Some times the whole limb could move, in other animals the lower leg or only the toes; in some, both hind limbs, in others only one regained the power to move. The results are extremely important, for they clearly show that if a minute part of the cord be left in the plexus region, subsequent changes suffice to permit of some leg movements, and conversely when the limbs have not moved for a long period, three or more months, this may be taken as presumptive evidence that all the motor nerve cells of the plexus have been destroyed. The experiments also show that a certain amount of healing and regulation of the cord takes place, but this matter will receive further treatment later.

No. 1.1 B. It was not intended at the time of operation that the cord in the plexus region should be injured. The tail stump was 2 mm. long. The cord was destroyed about 4½ mm. Both legs amputated. After the operation the left leg moved normally. The right however did not move at all, but within a few days

<sup>4</sup> The length of the tail was measured just behind the cloaca.

rather infrequent and jerky voluntary movements were observed. Sixty-one days after the operation both limbs moved readily. The explanation for the temporary paralysis of the one leg is found in the fact that the needle had penetrated that part of the cord whence the third plexus nerve on the right side takes its origin. The injury is confined practically to the one side. The cellular and fibrous layers and both the roots of this side are gone. The abnormally large lumen is eccentric. The injury, though decreasing anteriorly, extends slightly beyond the origin of the second plexus nerve. Anterior to this level only secondary degenerative changes, to be later described, are found.

In No. 1.37 the injury was far greater. Slight voluntary movements were not noted until 65 days after the operation. Examination showed that the nerve cord had been completely destroyed in the tail and that part of the plexus region from which plexus nerves II and III arise. Nerve I takes its origin in a very malformed and dilapidated cord, less than one-half its proper size, with the lumen in the form of a horizontal slit almost dividing the cord into an upper and a lower half, and the fibrous layer practically gone. We have here an example of the removal of the major portion of the cord from the plexus. The motor cells of nerves II and III were destroyed; most of the motor cells of nerve I were also destroyed. The surviving cells multiplied, grew together to form an irregular flattened ring, the cells of which supplied nerve I with some motor stimuli. Hence the vague movements of limbs after so long an interval.

No. 1.7, first moved the rear limbs 42 days after the operation. Examination revealed conditions essentially like 1.37.

No. 1.35 moved only after 125 days. Internally the injury and changes were like 1.37.

No. 1.20. Rear limbs began to move "hesitatingly" 74 days after the operation. Preserved 19 days later. The result is instructive because it demonstrates that the nerve cord at so anterior a level as the plexus has the power to repair an injury and to proliferate cord cells to a limited degree. The cord was normal anterior to the origin of the first plexus nerve. Posterior to this level the cord had been severely injured. It is shruken to about one-half

its proper size, is egg-shaped in section, with its long axis horizontal. The lumen is a mere slit. The fibrous layer has largely degenerated. In places the middle of the slit comes together to form two separate lumina, thus forming a double cord. A little farther caudad a clump of cells have formed a smaller dorsal nerve cord, which enlarges posteriorly. The ventral one only connects with the plexus nerves. The two finally fuse, the two lumina unite to form one, and slightly beyond both the cords disappear from the rest of the plexus region.

No. 1.25. The left limb moved slightly two days after the operation, and from this time on there was a steady increased ability to move the limb. By the 71st day it moved quite normally. The right leg however remained paralyzed.

Like 1.1 B, the cord of this animal was injured on one side only, and like 1.20 it clearly showed that a considerable degree of regulation in the nerve cord may take place. Plexus nerves II and III are cordless. Plexus nerve I takes its origin in a cord, the cells, fibers and roots of which have been destroyed on one side, namely, the right. The cord however has rounded itself out around an extraordinarily large lumen, eccentrically placed, and innervates the left leg, enabling it to move.

No. 5.2. The legs remained paralyzed for 117 days. During the next 10 days they moved slightly. The cord had been injured over an area beginning 6 mm. anterior to the plexus and extending almost as far as the origin of the second plexus nerve. Posterior to this region the spinal column was cordless. In the first mentioned area the cord is extremely degenerate, as indicated by the small diameter, vacuolized fibrous layer, and irregular shape and large size of the lumen. It ends in an irregular mass of minute degenerate cells. From these appearances we may infer that the cord had not been totally destroyed, but that a number of cord cells continuous or nearly so had been left. These have made abortive attempts to develop into a new cord. Under tail regeneration evidence will be brought forward for believing that while the cord can round itself and even grow laterally, i. e., grow nerve fibers, posterior regeneration at this level never takes place. The improvised cord can make enough motor connections with the limbs

to permit them to move slightly. It took 117 days for all these changes to take place. In the meantime the new leg had grown 2½ mm. long and had differentiated all the toes.

No. 1.13. Four days after the operation both limbs showed indefinite movements. The sole of the foot was upturned for a long period even after the leg was able to move considerably. The animal was preserved 130 days after the operation. Internally the parts were very much like those already described in 1.25. The cord had been injured but not totally removed from the region of the plexus. Of especial interest are the nerves of the leg. Quite different from those in the totally paralyzed legs of the same stage, the motor as well as the sensory nerves have regenerated and have grown to and beyond the amputated end. In point of fact these new fibers cannot he distinguished from normal ones both as to structure and staining power. The opposite limb amputated 39 days before preservation stands in sharp contrast, for typical Wallerian degeneration has set in near the cut end. The left hind limb had grown 5 mm. and was quite normal, the right had only grown 13 mm.

No. 14.1. The legs began to move after a lapse of 159 days, the left with greater difficulty than the right. The animal was preserved 177 days after the operation. The cord had completed itself from injured remnants left after the operation. Such regulation extended from nerve II posteriorly. Just as in the preceding many nerve fibers, mostly sensory, have regenerated to and beyond the amputated end. The new part of the leg was 3½ mm. long, but quite normal with 5 distinct and typically formed toes.

#### Control Series

We may now briefly consider the nature of the changes that take place after amputation of the hind limbs and without injury to the cord, and compare them with the changes after injury or removal of the cord.

The first animal to be mentioned was preserved 35 days after amputation (No. 10.4). The nerve fibers show near the cut end only the typical degeneration already described. The bone proliferated a cap of cartilage and between it and the skin there was a mass of "embryonic" tissue. The sections are not different from

similar sections of limbs of a corresponding stage of development, taken from paralyzed or partially paralyzed animals.

Another animal (No. 2.8) preserved 55 days after the amputation had regenerated a new limb 3 mm. long, bearing 4 toes. All the elements of the leg, foot and four toes were laid down. The nerves have degenerated farther proximally than in the preceding, and the rate, extent and kind of degeneration are quite the same as described under paralyzed and partially paralyzed individuals. In the pelvic region there was no trace of degeneration either of the cellular or the fibrous layers of the cord or of the nerves between the base of the hind legs and the lumbo-sacral plexus.

No. 15.4 is worth mentioning because the limbs bore no external sign of regeneration, although 50 days had elapsed since amputation. Internally the proliferation of cartilage and the formation of "embryonic" tissue had proceeded less rapidly than 10.4. The nerves were practically the same as 2.8.

In No. 2.8, the leg had grown 4 mm. long, with four distinct toes, during the 116 days after amputation. The nerves had degenerated still farther proximally, in fact close to the base of the leg. But of special importance are the extensive constructive changes whereby the regenerated nerve fibers have replaced in large part those broken down. This is about at the same rate as the regenerative changes in partially paralyzed animals, in No. 5.2 for example. The destructive changes did not affect the cord or nerves proximal to the base of the legs.

No. 2.10 is interesting, for though the regenerating period was the same as 2.8<sub>2</sub>, the nerves had degenerated from the amputated end only as far as the knee, not above it. Like the preceding the nerve fibers have grown back again towards the regenerated leg.

In 1.48 the limb has been cut off above the knee, and 184 days thereafter the animal was preserved. The new motor nerve fibers have largely replaced the degenerate ones and reached almost to the cut level, while the sensory fibers have penetrated to a considerable distance within the new limb.

The few instances above, but too briefly touched upon, and chosen from a large number, are perhaps sufficient to make clear an outline of the history of the nerves after ordinary amputation for purposes of comparison with other cases.

It might even be maintained from a study of the changes in ordinary amputated limbs only, that nerve impulses are not needed in the replacement of lost parts; for it was found that after amputation the severed nerves broke down near the cut end and continued to degenerate proximally; and that while these processes were going on, the bone proliferated cartilage, the old muscles regenerated new ones, etc.; and that only after the new leg had grown to a considerable size did the nerves grow into them. If the conclusion were based on this evidence of ordinary regeneration alone, it might be claimed that the facts did not show whether an initial stimulus—before the degeneration of the nerves had begun—may not have sufficed to start the regenerative processes. This criticism has, however, been thoroughly offset in the experiments of the previous sections on paralyzed and partially paralyzed animals.

### Dorsal Ganglia of Plexus Destroyed

Up to this point the removal of motor impulses alone was considered. Much time was given to this problem, because according to prevailing opinion the "morphogenic" and "trophic" influences travel or originate in the motor system. Herbst and others, however, believe that sensory stimuli exercise a controlling influence in regeneration. In this section we will examine the effect of removal of either the dorsal ganglia alone or the dorsal ganglia and the nerve cord.

While the cord may be readily removed, the destruction of the dorsal ganglia is not a simple matter. Each ganglion is encased in a bony compartment. Attempts to remove the ganglia by cutting out the spinal column led to the death of the individuals. The method finally adopted was to open one side (the left) of the anæsthetized animal in the region of the pelvis, expose the nerves that innervate the limb, trace them as near as possible to their origin and after destroying the protecting bone with a broach or hot needle, scrape the contents most thoroughly. The slit was then sewed and both legs amputated. The cord was destroyed in many animals either by breaking through the sides of the neural arches or, as in previous sections, by piercing the vertebral canal by way of the tail.

The injury to the nerves was followed by paralysis of the limbs on the operated side. Succeeding events depended upon the nature of the injury. If all the plexus nerves had not been severed there was a return of motor functions within a few days. When all were cut, the limb did not move for 30 or more frequently 60 days. In other animals where the nerve cord had been injured as well, both hind legs were paralyzed either permanently or temporarily. After successful operations, the limbs were insensible to strong stimuli, even though the motor nerves were intact, for the sensory branch of the reflex arc was destroyed. When sufficient time was allowed, both hind legs regenerated, the operated one considerably slower than its mate.

No. 12.57. By the 30th day after the operation the right leg had grown 2 mm. long. No toes had been developed; the left leg showed externally no regeneration. It was paralyzed throughout the experiment. Serial sections proved that the three dorsal ganglia of the plexus had been entirely destroyed on the left side of the animal. Not a single ganglion cell could be found. The broken bony capsule was vacant except for pigment cells and a few connective tissue cells. The cord had also been injured in this region. The fibrous layer throughout the plexus had degenerated, and the cord has shrunken to about one-half its typical size; the lumen is irregular and eccentric. Concerning the plexus nerves, not enough time had elapsed for the degeneration of the fibers to have taken place except near the amputated end of the right limb. In the left (operated) leg, as might have been expected, the nerves are far more degenerate, bordering on complete disintegration. In this animal all the sensory and probably all the motor nerve stimuli as well were prevented from reaching the amputated end of the leg. Unfortunately not sufficient time had been allowed to make certain whether the operated limb could or could not regenerate under these conditions. Within the limb merely a cap of cartilage had formed about the broken end of the bone shaft. The main question is thus left unanswered.

In other cases, though three or more ganglia were totally destroyed, only two of these were plexus ganglia, as in 12.36, 12.50, 12.51, etc. (Table 1). In some of these animals the cord had also

TABLE 1
Spinal Ganglia of Plexus Destroyed

No.	DAYS	AFTER	AMOU	NT R	EGENEI	INTERVAL BETWEEN OPERATION	GANGLIA TOTALLY	REMARKS	
	Operation	Amputation	Ri	ght	L	eft	AND FIRST	DESTROYED	** Marrier \$ 70 to 10
			mm.	toes	mm.	toes	days		
12.24	77	58	2 <del>1</del>	4	31	4	4		Recovered com- pletely*
12.25	77	58	11/2	•	1	0	4	;	Recovered com- pletely
10.3	110	110	+	į			11	i	
10.4	46	35	11		1	!	11	:	
10.5	123	113	6	5	6	5	11	!	
12.55	96				!	; ;	17	Second and third plexus ganglia.	Not amputated. Almost complete recovery.
12.41	51	51	33	4	2 1/2	4	20	g	Recovery com- plete.
12.35	65	57	4	4	0		38	1	Slow but com- plete recovery.
12.57	39	39	2	-	•		39	First, second and third plexus ganglia.	
12.59	39	39	•		•		39	First and sec- ond plexus ganglia.	Slow recovery.
12.60	39	39	3	0	0		39	First, second and third plexus gan- glia.	Complete paraly- sis.
12.61	88	88	6	5	+		39		Not paralyzed at
12.62	88	88	4	4	0	ĺ			Almost norm.
12.51	57	43	4	0	0		49	First and sec- ond plexus ganglia	Slow recovery
12.36	59	51	33	5	23	4	52	First and sec- ond plexus ganglia	Slight recovery
12.50	62	48	31	0	3	0	57	First and sec- ond plexus ganglia	Partially recovered

<sup>\*</sup> Recovered completely from paralysis of limb. Movement normal.

TABLE 1-continued

No.	Operation Amputation		AMOUNT REGENERATED				BETWEEN OPERATION AND FIRST	GANGLIA TOTALLY DESTROYED	REMARKS
10 1	148	78	mm. 4	toes	mm. 2	toes	days	First, second and third partially de- stroyed	Permanently par- alyzed
12.27 12.56 12.58 12.64	68 73 88 67	6 <b>4</b> 88	2 1 7	4	31/2	3			Permanently par- alyzed Permanently par- alyzed. Died. Permanently par- alyzed Permanently par- alyzed. Died.

been injured. Nevertheless a new leg developed on the operated side as well as the non-operated side.

No. 12.50. The left limb first moved 62 days after the operation, but not in any coördinated manner. The right leg had regenerated only slightly faster than the left, for the right was 3½ mm. long, the left 3 mm. long. Like 12.59 and probably like 12.51, but two of the plexus ganglia were totally destroyed, viz: ganglia I and II, yet the interval was sufficiently long to allow the limb to regenerate.

In No. 10.1 prese ved after 148 days the right leg regenerated 4 mm. and the left 2 mm. long. Ganglia I and II had been removed in toto, while ganglia III was only partially destroyed. The cord had also been destroyed in the region of the 1st and 2d plexus ganglia. Anteriorly and posteriorly the cord, while showing degeneration of the fibrous layer, does not appear to have been directly pierced. Fig. 4 is a cross-section through the 2d plexus ganglia. On the left side the ganglion has been wholly destroyed, together with its bony encasement. The cord together with the

greater part of the neural arch has likewise been destroyed. The latter however has been partly replaced by an irregular mass of cartilage, shaded lighter in the figure. On the right side the ganglion and its nerves and its bony compartment are intact.

Unfortunately in all cases where at least the three plexus ganglia were wholly destroyed the animals had been killed before a sufficient time had elapsed to determine whether regeneration could take place. All that the evidence warrants is the conclusion that

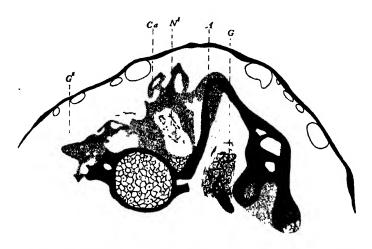


Fig. 4 Cross-section through region of pelvic plexus. Shows complete removal of the nerve cord and left dorsal ganglion. Cartilage has proliferated to partly fill space formerly occupied by left half of vertebra. Magnified same as Fig. 2. A, remains of neural arch; Ca, proliferated cartilage; G, sensory ganglion; G', space formerly occupied by ganglion; N, space previously occupied by vertebral canal.

the removal of the major part of the sensory stimuli, with or without the destruction of the motor stimuli, does not inhibit regeneration of the legs. Later, however, it will be conclusively demonstrated that in the tail of this animal dorsal or sensory ganglia do not provide the stimulus to morphogenesis.

#### Nerves Removed from Limb

Very little need be said concerning this series. The nerves of the thigh were exposed, and a rather large piece removed from one or both nerves, then the legs were amputated usually below the knee. We have already considered the objections to this method of preventing nerve impulses from reaching the amputated end, and it is hardly necessary to repeat them here. The limbs regenerated the missing parts as readily as the control animals. The changes in the nerves are of no special importance since they were essentially the same as those already described.

## Second Amputation of Paralyzed Limbs

Of far greater importance was the series of experiments in which after varying intervals following removal of the nerve cord the limbs were amputated a second time. A sufficiently long period, i. e., from 108 to 157 days intervened between successive amputations, for the motor nerves to degenerate completely, and along their whole length. The second amputation was made at the same or more proximal or more distal level than the first amputation. In every case there was complete paralysis throughout the experiment. Nevertheless typical legs were regenerated.

The result furnishes strong evidence that the stimulus for regeneration is not a nerve stimulus, or more correctly not a motor stimulus. In the animals of this series the motor cells as well as the motor fibers of the limb had been destroyed, the former directly, the latter through subsequent degeneration. The sensory fibers had also degenerated for considerable distance, and had not yet begun to regenerate, so that the possibility that the mere presence of the nerve fiber may have been the stimulating factor cannot hold. In conclusion it might be added that the limbs differed in no way from those in the control series in which the legs were also amputated a second time.

The legs of the following inidviduals were examined. The time between the original operation and the subsequent amputation of the leg is given in column 2, between this date and the last removal of the leg, in column 4.

TABLE 2

Paralyzed limbs amputated a second time

NO.	DAYS AFTER FIRST AMPUTATION	DAYS AFTER PARALYSIS	AMPUTATION	PARALYSIS
1.36	110	110	53	163
1.40	147	157	34	188
1.43	50	108	72	180
1.45	100	140	54	194
5.4	154	130	36	166
1.42	84	110	145	250

#### Other Attempts to Prevent Regeneration

Though not directly connected with the main issue, these experiments are perhaps of sufficient interest to warrant their insertion at this place. The effort was made to prevent the regeneration of the leg by the removal of some other non-nervous tissue. Tornier had found that lack of growth of the skeleton of the tail in salamander prevented further growth of the tail. Morgan observed that the removal of the notochord in frog tadpoles inhibited the regeneration of the tail. These observations suggested the removal of the bone in the leg. This was done in two ways. In the first place a piece of the femur about 2 mm. long was cut out from the amputated end. The distal part of the limb disintegrated and regeneration began at the more proximal level where all the tissues were intact. In the second place, a piece of the bone was removed from the middle of the femur, in the belief that regeneration might be inhibited until the two pieces grew together. But here too the peripheral end sloughed off, or disintegrated down to the level at which all tissues were present and continuous, whence regeneration began. In another group the leg was cut in such a manner that the femur protruded beyond the rest of the leg about I to 11 mm. Again the bone disintegrated to the level of the other tissues, then regeneration began.

We may refer here once more to Wolff's experiments. Wolff does not state explicitly how much of the nerve cord and dorsal ganglia were destroyed in each animal. Subsequent sensory and

motor reactions indicate very clearly that the whole of the cord within the plexus region had not been destroyed, for, in my experiments permanent paralysis followed this operation, and when a minute part of the cord was left in the plexus, the limb sooner or later displayed some movement. The presumption is then that in Wolff's experiments some motor stimuli were transmitted to the limb, from the very beginning or soon thereafter. It is thus highly probable that only a part, perhaps the major part, of the nerve stimuli had been withdrawn from the limbs in his experiments.

Because most of the limbs whose nervous supply has been partially removed had a reduced number of toes, Wolff was led to adopt the view that the nervous system exerted some influence in regeneration. But what is the nature of this influence?

Does the removal of the nervous system remove from the limb certain morphogenetic stimuli, thereby preventing the formation of new structures, or does its absence cause injurious secondary conditions such as changes in blood supply and therefore of food and oxygen, accumulation of excretory products, the degeneration of the musculature and other tissues, etc., and thus indirectly affect the regeneration of the limb? That loss of nerve stimuli brings these injurious conditions about is well known. That it should tend to the formation of incomplete or malformed organs during regeneration is no more to be wondered at than that malformed structures should result from injuries not directly involving the nervous system or from the use of various chemicals in embryonic development. The morphogenetic processes go on in the absence of nerves, and in spite of the adverse conditions, as witnessed in the normal differentiation of cartilage, muscles, etc., of the leg, foot and tail. The surprising thing is not that organs are sometimes atypic, but that regeneration should take place at all under the circumstances.

## Regeneration of the Tail after Removal of the Nerve Cord

Diemyctylus, like other newts and salamanders, readily regenerates its tail. About twenty-five days after the amputation, the time varying largely with the temperature, the bud of the new tail

makes its appearance. Externally the regenerated tail can be distinguished from the stump by (1) its color—it lacks the brilliant spots characteristic of D. viridescens, and by a general dull slate background not typical of the rest of the animal; (2), its size—it is perceptibly thinner and smaller dorso-ventrally, and a distinct depression marks the limit of the old tail, at least in the early stages; (3) its softness due to lack of ossification of the skeleton. Internally the regenerated area is characterized by the presence of embryonic or incompletely developed musculature—especially in the early stages—by the cartilaginous skeleton, by the large masses of undifferentiated tissue in the dorsal and ventral parts of the tail. It is seen that both externally and internally there is a more or less clearly marked division between the old and the regenerated areas, and the distinction becomes less as the new parts grow longer. The nerve cord, however, rarely shows such a clear separation of the two parts.

The removal of the cord was a relatively simple matter as already described, but the complete extraction of the spinal ganglia was difficult and uncertain. I shall first deal with motor influences, leaving for later consideration the study of the sensory stimuli.

A number of normal tails were first examined in serial sections. A reconstruction of sections of the tail showing the approximate distribution of the nerves is partly seen in Fig. 1. The innervation is quite the same throughout the tail. The spinal column extends to the very end of the tail. Each vertebra encloses a pair of spinal or dorsal ganglia, through which the dorsal and ventral roots pass. These unite beyond only to branch again, one passing dorsally, the other ventrally. Many smaller branches are given off from these. The point of especial importance, however, lies in the fact that the nerve fibers extend only into the region of the next two or three vertebræ, and not further distally. In other words, the nerves at any level have their origin only in one or more of the next three segments, i. e., about 4 to 4½ mm. anterior to any level. It follows that the removal of the source of motor impulses from the amputated end depends on the complete destruction of the nerve cord for at least 5 mm. anterior to the cut end.

Destruction of Less than 5 mm. of the Nerve Cord

The operation was the same as that previously described. The cord was removed from 2 to 4½ mm., by a needle, or glass capillary or, better still, by the broach. In nearly every instance—where sufficient time was allowed—a new tail regenerated. It was somewhat slower in making its appearance than in the control animals, but, having once appeared, further growth was quite normal. Here too it is very probable that the injury to bones and surrounding muscles, rather than any direct effect of the lack of nerve supply, is responsible for this retardation. It was pointed out in a preceding section that under certain conditions disintegration of the wounded end often took place. This possibility was kept in mind and unless specifically mentioned to the contrary all tail stumps measured at the time of fixation exactly the same as after the operation. There sometimes appeared a discrepancy of \(\frac{1}{2}\) to \(\frac{3}{2}\) mm., but this was probably due to the contraction of the tissues at the wounded end.5

The following six animals however showed no external sign of regeneration, though 30 to 91 days had elapsed after the operation.

No. 1.1 B. The tail was amputated close to its base, then 4 mm. of the cord was destroyed with the needle. After 30 days no regeneration had occurred.

Between 5 and 4 mm. from the end, the cord was normal in size and shape. The fibrous layer however had degenerated and disintegrated, giving to the cord a very vacuolated appearance, one-half of which is shown in Fig. 5. The cellular or neuroglia layer has been greatly decreased, due to the degeneration of many of the cells. Just posterior to this level the cord suddenly shrinks to about one-half its normal diameter, and becomes very misshapen. The dwindling proceeds to within 2 mm. from the amputated surface, at which place the cord ends as an irregular clump of degenerate cells. Without going into further detail, it may be said that there is every evidence that the needle has merely injured the cord between the 4th and the 2d mm., while between the

<sup>&</sup>lt;sup>5</sup> All measurements are made anterior or posterior to the amputated level.

2d mm. and the cut end the whole of the cord was destroyed. Reparative changes of the cord not unlike those mentioned in connection with partially paralyzed legs have taken place. The point of especial interest is the absence of the cord from the end coupled with the lack of regeneration.

In this particular animal there was a peculiar growth of cartilage, at the injured end, which forming an irregular mass practically plugged the end of the neural arch. This plug it might be sup-

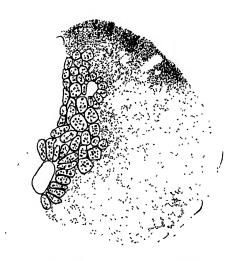


Fig. 5 Cross-section of nerve cord a short distance anterior to amputated level. Illustrates extent of degeneration anterior to cut end. Cellular layer reduced. Fibers disintegrated most completely in the ventral or motor parts.

posed may have had something to do with the lack of regeneration of the tail.

No. 1.11. Tail amputated near the distal end; 4 mm. of the nerve cord removed. After 32 days no regeneration. The nerve cord was normal as far as  $5\frac{1}{2}$  mm. from the end. From this level to about  $4\frac{1}{2}$  mm. the myelin of the fibrous layer shows an increasing granulation. At the latter level the cord looks not unlike Fig. 5. From this point to about 1 mm., there is a marked shrinkage. The disintegrated and vacuolated fibrous layer has given

way to a narrow compact layer which in turn disappears close to 1 mm. from the end. The cord is here reduced to a mere ring of neuroglia cells, enclosing a large lumen, Fig. 6. Beyond this level there is no cord.

That the broach has extended as far as 4½ mm. from the end was shown by the cartilage that has proliferated from the injured inner lining of the neural arch, almost completely filling the space

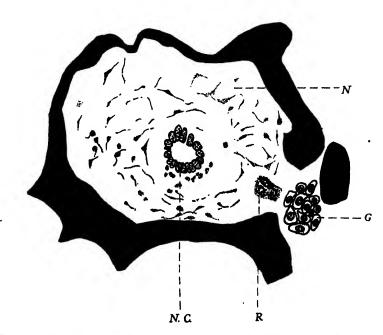


Fig. 6 Cross-section near posterior end of nerve cord, at early stage of regeneration. The cord consists of a single layer of cells. N, almost empty vertebral canal; NC, regenerated end of nerve cord; G, part of sensory ganglion; R, proximal end of roots of the cord.

between the arch proper and the diminutive cord. It will be remembered that this broach was as great or greater in diameter than the arch, so that the spirally wound teeth often destroyed not only the contents but injured the arch as well. The degenerate ventral roots about 2 mm from the end were almost united by strands of connective tissue, and over them and separated from

them was the newly regenerated nerve cord. Here also after thirty-two days, or more than sufficient time for regeneration to have taken place, only the cord had regenerated, but not quite to the amputated level, and the absence of the cord from the end is again associated with the absence of the new tail.

No. 16.8. Removed the cord from the last 4 mm., no regeneration after 45 days.

There was far greater injury to the bones of the vertebræ and to the muscles than in the preceding. The centra and hæmal arches were almost completely destroyed. The cord, like the preceding, had regenerated, but only to within 2 mm. of the end. The far greater injuries to surrounding tissues may account for this retardation. However this may be, the absence of the cord is again associated with the failure to regenerate the tail.

No. 16.9. Destroyed the cord from the last 4 mm., no regeneration after 61 days.

Degeneration of the fibrous layer has proceeded farther cephalad than in any of the preceding animals, extending from about 4 mm. to the 7th mm. The new cord has grown as far as the amputated end. Near the 4 mm. level, the inner lining of the arch has proliferated much cartilage. Distally however the neural arch, the centra and the hæmal arches were largely destroyed, leaving mere fragments of bone. These have proliferated cartilage that cemented many of the pieces together. Near the tip of the tail practically the entire skeletal structure was missing.

More than twice as much time has elapsed than is ordinarily required for the new tail to appear. During this interval the new nerve cord has actually reached the amputated end.

No. 16.10. Operation and time the same as the preceding. Like it, the fibrous layer of the cord had degenerated about 2 mm. cephalad. The very attenuated new cord had grown to and slightly beyond the cut end, into the cap of embryonic tissue. Like the preceding, the skeletal axis is practically gone from the end. Tail had not regenerated.

No. 16.2. The cord was destroyed from the last 3½ mm. Regenerated a bud almost 1 mm. Time, 92 days.

Degeneration of the fibrous layer of the cord has proceeded over

3½ mm. Near the end of the old cord the fibers have disintegrated completely. The new cord extends almost to the tip of the tail. The neural arch had been injured but only slightly, resulting in the characteristic proliferation of cartilage inside the arch near its distal end. The end bud gives the impression that a new tail was about to develop.

The next seven animals were chosen from a very much larger number which, though operated in the same manner as the preceding, nevertheless grew new tails, in some cases as large and complete as the control animals.

No. 16.14. Tail amputated about the middle. Three millimeters of the cord destroyed. Sixty days later the new tail had regenerated 4½ mm. long.

Actually 23 mm. of the cord was destroyed, thereby destroying the cord cells from the distal two pairs of spinal nerves would not be enough to prevent some motor innervations to the cut surface. Subsequently the cord degenerated anteriorly about 13 mm., which may have and probably did remove the source of all the motor stimuli to the end. Yet in this case and in those to follow a new tail appeared, provided the skeletal axis and nerve cord were present at the amputated level. The nerve cord in the sections showed a gradual shrinkage posteriorly. The vacuolated fibrous layer is succeeded by a layer of embryonic fibers. neuroglia cells increase in numbers and migrate laterally in each vertebra, to form the two primitive dorsal ganglia, and posteriorly to constitute the regenerated cord, Fig. 7. No clear line separates the old from the new cords, yet they are quite different. were no medullated fibers in the regenerated tail. Concerning the musculature and skeleton we need not enter into details here. It may suffice to state that the old muscles give rise to the new, that the old bony vertebræ regenerated the cartilaginous skeleton of the new tail, that the parts of the latter, growing at different rates, produce first the centra and then the simple bars that constitute the neural arch, and lastly the hæmal arch.

No. 15.6 differs so little from the preceding that the account there given will substantially serve for this animal.

No. 15.1. This animal regenerated a tail 5 mm. long. It differs from the preceding two animals as follows: (1) 4½ mm. of the

cord was destroyed instead of 2\frac{3}{4} mm.; (2) the .time was greater, namely, 78 days; (3) there was greater injury to the neural arch and a corresponding greater proliferation of the cartilage.

No. 1.4. The needle destroyed but 2 mm. of the cord. For the next millimeter, the cord had been removed on one side only. The subsequent changes were the same as those already described. Four millimeters of the tail had regenerated in 66 days.

No. 1.9. Destroyed 3½ mm. of the cord. After 124 days the new tail was not quite 3 mm. long. Degeneration of the fibers of the cord had extended as far as 8 mm. from the amputated level. No medullated fibers were present in the distal 11 mm. of the tail.



Fig. 7 Cross-section. Later stage of regenerated nerve cord, showing multiplication of cells and their migration laterally to constitute the ganglia, and the layer of embryonic fibers. Figs. 5 to 7 incl. same magnification.

No. 1.3. Destroyed 5 mm. of the cord. Regenerated tail 6 mm. Time, 142 days.

No. 1.8. Destroyed 4 mm. of the cord. Regenerated tail 6½ mm. Time, 170 days. It was rather surprising to find that even as late as 170 days after the operation there were no medulated fibers near the amputated end nor in the 3½ mm. anterior to it. This of course is not surprising when taken in connection with what was learned concerning the slow rate of regeneration of the cord and fibers of the leg. Further progress consisted in increased length of the tail and increased differentiation of the different tissues.

#### More than 5 mm. of the Nerve Cord Removed

As much as 7 to 25 mm. of the nerve cord were destroyed from different levels of the tail and lumbar region of different individuals. The time varied from 17 to 177 days.

No. 1.19. Amputated distally; 9 mm. of the cord destroyed. Preserved 17 days later. The cord was quite normal until 9 mm. from the distal end, where the fibrous layer had begun to degenerate. Between 9 and 6 mm. from the end there is no distinct cord, merely scattered groups of broken down cells. Between 6 mm. and the end no trace of the cord could be found. This case shows that within 17 days degeneration of nerve fibers in the cord had already taken place.

No. 1.16. Time, 25 days. The distal 8 mm. was preserved and showed not the slightest trace of the nerve cord as in Fig. 2.

No. 14.14. Tail stump, 30 mm. long; 7 mm. of the cord destroyed. Time, 36 days; no regeneration.

A very diminutive cord extends from the 9th to the 6th mm., beyond which there is no sign of the cord. Regeneration of the new cord has taken place for a short distance in the manner briefly described in the preceding section.

No. 1.1 A. Tail stump, 25 mm. long. Needle pierced 12 mm. of the cord. Time, 38 days.

Only few fibers have degenerated anterior to the 12 mm. Between this level and 11 mm., considerable degenerative changes have taken place in both fibrous and cellular layers of the cord. Reparative changes have also set in. The neuroglia cells have multiplied greatly to form a double cord about 9 mm. from the end. Slightly posterior to this the vague outline of a third cord is found. Just beyond this point the two main cords fused together, and then with the third cord disappeared completely, leaving the distal  $8\frac{1}{2}$  mm. cordless.

The needle which had been used in operating had but broken or crushed the cord into fragments, some of which proliferated new cells to form the double and triple cords. The reparative changes are essentially like those discussed under "Partially Paralyzed Animals." In the following three individuals no trace of the cord was found in the operated regions of the tail.

No. 14.19. 9 mm. of the cord destroyed. Time, 62 days.

No. 14.2. 19 mm. of the cord destroyed. Time, 97 days.

No. 15.3. 6 mm. of the cord destroyed. Time, 101 days.

In 14.8, 11 mm. of the cord were removed. Preserved after 156 days. The cord contained a degenerate fibrous layer anterior to the 11th mm. Posterior to this level the cord was double until the 10th mm. Beyond this there was no cord whatsoever.

The next three animals showed unmistakable evidence that the nerve cord had regenerated, towards, but not quite to the amputated end: No new tails developed.

No. 14.18. Amputated about the middle of the tail. Destroyed 7 mm. of the cord by means of a glass capillary. Time, 124 days.

Anterior to the 9th mm., the nerve cord was quite normal. From this level, extending to 1 mm. from the end, there was a tapering nerve cord that corresponded in every detail with regenerated cords already described. The fibrous layer had degenerated from the broken end anteriorly about 2 mm., and a new cord had regenerated posteriorly about 8 mm., during the 124 days.

No. 14.1. Stump, 11 mm. long. Time, 177 days. The cord was normal until 10½ mm. from the amputated end, where the cord becomes considerably thinner, and gives rise to the new cord. This becomes still more diminutive posteriorly, and ends altogether about ½ mm. from the amputated end.

No. 1.15. Stump, 20 mm. long. Destroyed 8 mm. of the cord. Time 126 days.

The cord was continuous to the very end of the tail, i. e., to the amputated end. The changes between the old and the new cord were so gradual and subsequent regeneration had concealed the traces of former degeneration so completely, that it was quite impossible to state where one began and the other ended.

If enough time be allowed and if circumstances be favorable we might expect that the nerve cord would reach the amputated end and thus permit regeneration to take place. This actually occurred.

In 15.4, 8 mm. of the cord was destroyed. Time, 72 days. The regenerated cord extended from about 8 mm. to the amputated end. All tails form a cap of "embryonic" tissue over the amputated end. In this animal also, the cap had formed, but was

so very large that it gave the impression from an external examination, of a regenerated tail, and in fact was so designated in my notes. Examination showed however that this cap contained neither cartilaginous skeleton nor nerve cord, and can therefore hardly be called a tail. The tail was in all probability about to develop.

In 14.21, 8 mm. of the cord was removed and in 79 days a new tail had actually developed 3 mm. long.

Anterior to the 10th mm. the nerve cord was normal. Between this and 8½ mm., the fibrous layer had degenerated, and the cord in this region håd shrunken considerably. The cord thins very gradually to and beyond the amputated end to the very tip of the regenerated tail. Here too, no sharp line can be drawn between the old and the new cords. Neither in this nor in any of the preceding animals were medullated fibers differentiated in the injured region. The neural arch proliferated cartilage, that cemented broken pieces together and formed irregular masses within the arch, and in this animal grew distally to constitute the skeleton of the new tail.

In more than fifty other animals the cords of which had been removed from the tail region alone, or from the tail and lumbar regions, the cord was found in every instance, on subsequent examination, completely absent from the neural groove of the operated area, and in every case a new tail failed to develop. Similar reparative changes already alluded to were observed, such as the formation of multiple cords, etc.

In the following table a number of the animals are arranged according to the extent of the regenerated nerve cord.

We might assume that the diminutive new cord, quite embryonic in character, transmits nerve impulses either through embryonic fibers too minute to be detected or through the protoplasm of surrounding tissues, and that this nerve stimulus induces regeneration. If this be so, it is quite difficult to understand why it is that these impulses do not suffice while the cord is growing toward and ever so close to the amputated end. But as soon as the cord actually reaches the end, regeneration begins.

TABLE 3
Regeneration of tail in Diemyctylus viridescens

No.	LEVEL OF	LENGTH OF CORD RE- MOVED	TIME	TAIL REG.	NERVE CORD REG.		
		mm	days	mm	mm		
16.14	middle	4	60	41	8½		
15.6	middle	4	66	31/2	71/2		
1.4	posterior	4	67	3	7		
15.1	middle	4	78	5	9		
1.9	posterior	31/2	124	2 1/2	6		
1.3	middle	5	142	11	61/2		
1.8	anterior	4	170	6	10		
15.4	?	8	59	2+	10		
14.21	posterior	8	74	3	11		
1.15	?	8	126	+	8+		
15.4	1	8	72	1	9		
16.10	middle	4	61	0	To amputated end		
16.9	1	4	61	•	To amputated end		
16.2	middle	4	92	0	Almost to amputated end		
16.8		4	45	•	Within 2 mm. of amputated end		
1.11	posterior	4	32	0	Within 11 mm. of amputated end		
1.1B	anterior	4	30		No regeneration. Reparative changes only		
14.1		101	177	0	10 mm., i. e., ½ mm. from end		
14.18	posterior	7	124	0	6 mm., i. e. i mm. from end		
14.8	middle	111	156	0	1 mm. Double cord		
14.14	posterior	7	36	0	I mm.		
1.1A		12	38	0	Reparative changes Double cord.		
1.19		9	17	•	•		
etc.			•	0	•		

### Cord Prevented from Reaching the Cut Surface

The matter was experimentally tested in the following manner. After removing 2 mm., or less of the nerve cord, the end of the neural tube was closed with a plug of paraffin or celloidin. Sometimes a flap of skin, like that of a pocket, was sewed over the wound to prevent the plug from falling out. In this experiment only a small part—if any—of the nerve supply to the amputated surface was destroyed. The elapsed time was more than sufficient to allow even severely injured animals to regenerate, viz: 204 days,

and though all the controls regenerated tails 8 to 11 mm. long, not one of the operated animals developed a tail. To make certain that lack of regeneration was not due to any peculiarity of the individuals, the tails of the operated animals were cut off a second time close to the former level, and new tails developed in the ordinary manner, and in the ordinary time.

#### Cord and Skeleton Removed

In another series of animals, both the cord and the major part of the skeletal axis were removed from the posterior two or three mm. Sometimes the end disintegrated down to the level where all the tissues were intact. In some cases no sloughing or disintegration took place, as in the following examples.

In No. 17.3 for instance there was no regeneration. Time, 77 days. No cord and only small fragments of bone were found in the distal 2½ mm. The cap of embryonic tissue had formed as usual, but no further constructive changes were visible.

In other animals of which 17.1 is an example, regeneration did take place. The operation was exactly the same as in the preceding animals. In 17.1 the time was also 77 days. Yet a new cartilaginous axis and a new nerve cord had grown to the amputated end and thence beyond to form a new tail 9 mm. long. In these cases it is very probable that not enough of the skeleton had been removed, so that they are essentially like those previously considered wherein less then 5 mm. of the nerve cord had been removed.

### Influence of Dorsal Ganglia in Regeneration

There remains the possibility that the spinal ganglia may play an important rôle in regeneration. Some doubt may be expressed with regard to the influence or lack of influence of the dorsal ganglia in the regeneration of the hind limbs. But with regard to the tail, it is perfectly clear that these ganglia exert no influence in regeneration. In those experiments where more than 5 mm. of the cord was destroyed, the ganglia were intact yet were unable to

incite the regeneration of the tail. In the series wherein the vertebral canal was plugged at the end, the ganglia were undisturbed, yet here also no regeneration took place. In the last series the presence of the ganglia did not stimulate the formation of the tail, when the essential factors were not present. Finally in a small number of animals the ganglia on both sides, together with the cord were destroyed for about 5 mm., yet the regeneration of the tail was not inhibited. In other words sensory stimuli are not responsible for the regeneration or lack of regeneration of the tail.

#### Summary.

The limbs. Having determined the course of the nerves in the rear limbs and tail, the animals were operated in such a way that the nerve cord was completely destroyed in the tail, in the lumbosacral plexus and from one to six vertebræ anterior thereto. Study of serial sections established the equally important fact that at the level of the plexus no regeneration of the nerve cord took place. In consequence the rear limbs were permanently deprived of all motor innervation. In a number of other animals the dorsal or sensory ganglia of the plexus nerves were likewise destroyed. The limbs were amputated either before or immediately after the removal of the cord or after intervals sufficiently long to permit the nerves of the leg to degenerate more or less completely.

Special attention was given to the nerves of the leg. Degenerative changes took place very slowly, about 150 days being required for the complete degeneration of the motor fibers. These were never regenerated. The sensory fibers however—where the dorsal ganglia had not been destroyed—degenerated but for a short distance from the amputated end, and then grewdown into the regenerated leg.

In a number of animals the legs were able to move more or less completely, but in every such instance at least one of the plexus nerves was found connected with the nerve cord. In no case was there any movement after the whole of the cord had been destroyed from the plexus region.

Regardless of the nature of the operation, and regardless of the subsequent degeneration of the various tissues (nerve fibers in-

cluded), the missing parts of the leg were readily regenerated in almost every instance.

The elements of the leg, foot and toes were differentiated in a perfectly typical manner. Certain malformations made their appearance and these were perhaps more numerous than among the control animals. But these were due in largest part to mechanical stresses or other indirect effects of paralysis. Many of the paralyzed legs, even at a late stage, could not be distinguished from controls of the same degree of differentiation.

The tail. The facts appeared at first to point to the conclusion that regeneration of the tail in Diemyctylus viridescens is directly dependent on motor innervation of the amputated end. This conclusion is contrary to that obtained in the regeneration of the limb of this animal, in the tail of the tadpole, and in the head of the earthworm,—to be hereafter described—and it seemed to follow either that the rôle of the motor nerve system was different in different parts of the same organism, and different in different animals, or, that there were some other factor or factors in the regeneration of the tail of the newt that I had not yet discovered. A detailed study of many more animals showed that the first alternative was wholly untenable.

It was found that at each level the normal tail is supplied by nerves that take their origin in the cord and dorsal ganglia of the next two or three anterior vertebræ, i.e., a distance of about 4 to 4½ mm. From this it followed that the destruction of less than 4½ or 5 mm. of the cord removes only a part of the motor supply while the removal of more than 5 mm. prevents all motor impulses from reaching the cut surface. Generally speaking the destruction of less than 5 mm. of the cord did not prevent the regeneration of the tail, destruction of more than 7 mm. nearly always inhibited the development of the tail.

After the operation, the end of the cord nearest the amputated surface undergoes degenerative changes of both fibrous and cellular layers. The changes cease and are superseded by constructive changes whereby either abnormal structures such as multiple cords are formed, or a new cord is developed. The rate of regeneration of the new cord varies considerably with the extent of the

injury to surrounding tissues and with the extent of the nerve cord removed. The cord may regenerate at all levels, except near the lumbar region, regardless whether 2, 4 or 14 mm., etc., were removed. We may omit the details concerning the regenerating cord in order to emphasize the fact that when a few millimeters were abstracted, the new cord soon grew down to the amputated level and a new tail appeared. In 6 animals however the cord had not grown to this level, though the elapsed time between operation and preservation was two to three times greater than that required for the tail to regenerate in control animals, and not one of these had developed a tail. Likewise in the series wherein 7 to 20 mm. of the cord were destroyed, a new cord grew in many animals towards the amputated end, but in no case did the tail appear until the cord actually reached the end. In one animal at least this had taken place and a new tail developed.

It is almost certain that after 2, 3 or 4 mm. of the cord are removed, subsequent degeneration prevents any motor stimuli from reaching the cut surface, at least during the early stages of regeneration of the new tail. There is also very good reason for believing that even after ordinary amputation the degenerative changes in the cord anteriorly prevents innervation of the cut surface.

The determining factor in the formation of a tail is not whether the cut surface is or is not supplied with functional motor nerves, but whether the nerve cord is present at the amputated end. If it is not present neither the absence nor the presence of functional motor and sensory stimuli suffices to induce regeneration.

The problem is further complicated by the presence of another factor, that determines whether regeneration shall or shall not take place. In a number of the animals the operation had been so severe that not only the cord but practically the whole of the skeletal axis near the end was destroyed. In every such instance regeneration was inhibited even though the nerve cord was present at the end. As soon however as a new skeleton grew to the end, and provided that the cord was also there, the new tail began to develop.

The matter was further tested by preventing the nerve cord from reaching the amputated surface, yet at the same time practically

without interfering with the innervation. The operation was performed in several ways so as to offset the possibility of the result being due to faulty technique. In every animal so operated, regeneration of the tail was absolutely stopped, at least during the 204 days of observation.

That the sensory ganglia exercised no stimulating or retarding influence was readily proved first, by completely destroying the sensory ganglia of the plexus nerves of the leg as well as the ganglia of the tail. The organs were invariably replaced. Regeneration could not be prevented. Secondly, by leaving these ganglia intact but preventing regeneration of the organ by other means. Under these circumstances the ganglia were unable to induce regeneration.

From these experiments it is clear that we no longer have any justification for referring to "morphogenic" or "trophic" or "formative" stimuli exerted by the nervous tissues in the regeneration of this adult animal, any more than the use of such expressions with reference to bony or cartilaginous tissues. In other words the adult D. viridescens replaces its tail and hind limbs without the aid of a nervous stimuli, either motor or sensory.

#### REGENERATION OF THE TAIL OF THE FROG TADPOLE IN RELA-TION TO THE NERVE CORD AND NOTOCHORD

Many experiments have been made upon amphibian larvæ for the purpose of ascertaining whether the central nerve system exercises a morphogenetic influence in development and regeneration. With few exceptions these experiments were made upon tadpoles in their earliest stages of development. Schaper, Rubin, Barfurth, Goldstein, Harrison, Braus and other investigators were led to conclude that development and regeneration of embryos take place independently of a central nerve system. Morgan and Davis '02, used older tadpoles. When they removed the nerve cord from the tail, regeneration proceeded as in normal individuals, but when a piece of the notochord was removed regeneraton was effectually prevented. In other words the nerve cord was incompetent alone to induce the formation of a new tail. It is condi-

Kölliker had pointed out—though not known to the writer at the time of experimentation—that the distal region of the tadpole tail was supplied by nerves that take their origin in the anterior portion of the tail. Under such circumstances the removal of the nerve cord need not and probably did not remove nerve stimuli from the amputated surface. Morgan and Davis are not explicit with regard to the levels at which their operations were made, nor how much of the cord was removed in each animal.

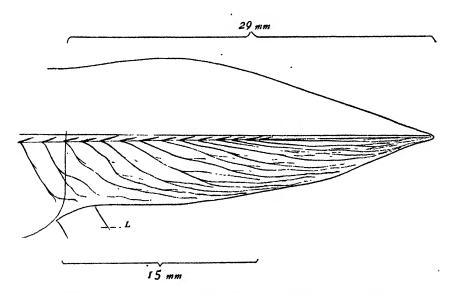


Fig. 8 Distribution of the nerves in the tail of the tadpole of a frog. Semi-diagrammatic. The nerves arise in the anterior half of the tail. The dorsal ganglia also limited to basal half of the tail. Length of tail, 29 mm. Sensory ganglia extend over anterior 15 mm, only. L, leg.

The following experiments were undertaken to re-examine these questions. A number of normal tadpoles were preserved in Hermann's fluid, and cut in serial cross-sections. The distribution of nerves in the tail was found to differ radically from the nerve distribution of Diemyctylus. In the latter each vertebra of the tail from the base to the tip, encloses a pair of dorsal ganglia and

gives rise to a pair of nerves that innervate the region occupied by the next two or three posterior vertebræ. In the tadpole however, though the cord extends almost to the tip of the tail, the dorsal ganglia and the paired nerves originate in the proximal half of the tail, Fig. 8. In the tadpole from which Fig. 8 was reconstructed, the tail was 29 mm. long, the ganglia were only in the anterior 15 mm., leaving the last 14 mm. without ganglia. The nerves in the body proper are segmentally arranged as in Diemyctylus, but in the tail the nerves grow more and more posteriorly so that the anterior region of the cord innervates the posterior parts of the tail.

Therefore in order to remove nerve stimuli from the amputated end, it becomes necessary not only to remove the cord and dorsal ganglia near the end but practically from the whole tail.

Various efforts were made to perform such an operation. The broach was used as in the newt experiment. But as there is no bony neural arch to guide the broach, it was largely a matter of chance whether the whole of the cord was removed or not. Another more satisfactory method consisted in slitting the side of the tail, exposing the notochord and overlying nerve cord, and then to cut or burn out the latter. Other methods, while they succeeded in removing the whole of the cord involved so much injury that the animals died.

After the effects of the anæsthesia, the animals swam around moving their tails considerably. Externally it was impossible to make certain whether these movements were due to motor activity in the tail proper or resulted from movements in the base or body region. New tails appeared on both control and operated animals. Many of these were examined in serial section. The marvellous power of repair together with the simple character of the cord, make it almost impossible at times to state with certainty whether a cord is a regenerated one, or an injured cord that has completed itself, or the original non-mutilated cord (in distal regions). One example may serve to illustrate the essential points:

No. 17. Length of tail 37 mm., removed 25 mm., broach used to destroy the cord. 35 days later the new tail was 6 mm. long.

Measuring from the amputated end, the nerve cord was appar-

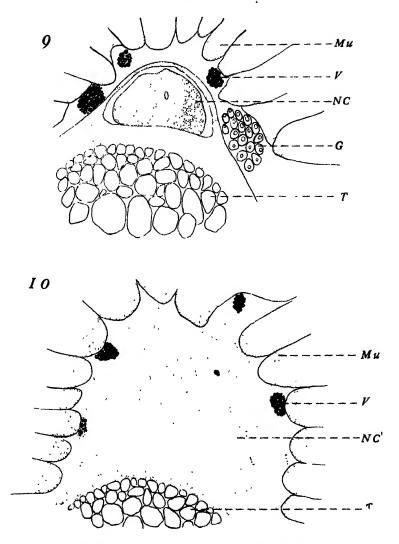
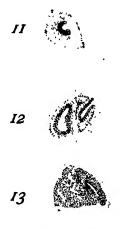


Fig. 9 Cross-section showing normal relation of the parts in the tail of the tadpole. G, sensory ganglion; Mu, musculature, much injured in Fig. 10; NC, nerve cord; NC', space formerly occupied by nerve cord; T, notochord; V, nerves.

Fig. 10 Cross-section showing complete removal of the nerve cord and severe injury to the musculature. Letters same as above.

ently uninjured anterior to 7½ mm., Fig. 9. Between 7½ and 6½ mm. the nerve cord was badly mutilated. In the next 2 mm. not the slightest trace of the cord was found, Fig. 10. The following 2 mm. however contained many mutilated pieces of the cord. For a short distance a crescent shaped mass of neuroglia cells is all that represents the cord, Fig. 11. These figures are all drawn to the same scale. A little farther there is a second diminutive sector of a cord which farther on, fusing with the first, forms an irregular double cord, Fig. 12. These end rather abruptly, but just before doing so four cords are seen almost fused together,



Figs. 11 to 13 Same magnification as Figs. 9 and 10. Represents abortive attempts of broken pieces of the nerve cord to round out and complete themselves.

Fig. 13. The concluding 2 mm. is absolutely cordless like Fig. 10. The regenerated tail throughout its length is likewise cordless. The mutilated cord between the two cordless areas gives rise to no nerve fibers nor to ganglion cells. Some dorsal ganglia had been totally destroyed, others only partially injured. The peculiar fragments and multiple cords, in all probability, represent pieces that had been severed at the time of the operation, and which had more or less successfully rounded out. No nerves however take their origin in these cords and no fibers are found in the regenerated tail. Other tissues, such as notochord, musculature, etc., were

often badly injured or destroyed, as seen in Fig. 10. But this did not appear to have any effect on the regeneration of the tail.

The other 14 animals examined differ in no radical respect from No. 17. They showed greater or less injury to the surrounding tissues, or more or less extensive injuries to the cord, or spinal ganglia. Yet in no case could I satisfy myself that the whole of the nerve cord had been removed from the entire tail stump, and that therefore the whole of the nerve innervation, either motor or sensory, had been removed.

There can however be no question but that regeneration can take place when the greatest part of the cord and spinal ganglia are destroyed and in all probability after all the nerve influences have been removed. The experiments furthermore corroborate Morgan and Davis's conclusion that a new tail may be formed in the absence of the nerve cord from the amputated end.

# REGENERATION OF THE HEAD OF THE EARTHWORM (LUMBRICUS) IN THE ABSENCE OF A NERVE CORD AT THE CUT SURFACE

The best example in support of the view that the presence of the nerve cord is the essential factor in regeneration, is that given by Morgan for the earthworm. Morgan ('02) removed the anterior segments of the earthworm and then cut out, from the ventral side of the amputated end, a narrow strip including skin, muscle layers and nerve cord. After a sufficient time had elapsed there appeared a new head, but only at the posterior end of the strip, i. e., from the end containing the nerve cord. No head regenerated at the amputated end, due, as Morgan believed, to the absence of the nerve cord. A modification of this experiment served to strengthen this conclusion. A similar strip or "window" was cut out, not from the amputated end, but beginning several segments posterior to this end. As Morgan had anticipated two heads were produced, one at each anterior cut end, where the nerve cord was exposed.

Joest and later Rabes were led to a similar conclusion as a result of their observations upon grafted worms. When the ventral nerve cords were free to come to the surface a head was regenerated at that point. Nusbaum ('08) recently made some very

interesting experiments along the lines laid down by Morgan, and came to a similar conclusion. He used Nereis diversicolor and instead of operating in the head region, he cut a strip, including nerve cord and muscles, near the posterior end of the animal. In others he destroyed the cord and surrounding tissues with a hot needle. Autotomy of some or all of the injured segments took place so that the new tail grew from a more anterior level. Careful examination was made of those animals wherein at least I to 3 cordless segments were left at the posterior end of the animal. He found that some time after the closing of the wound, fibers grew from the old cord toward the amputated end, and that the new tail did not regenerate until these fibers had reached the end a d supplied it with the proper nerve stimulus. Then a rapid proliferation of ectoderm cells took place anteriorly, which, with the probable addition of cells from the old cord, provided the material for making of the new nerve cord. In the author's own words: "Die mikroskopische Untersuchung zeigt uns, dass auch hier der Einfluss des Centralnervensystems keineswegs als ausgeschlossen betrachtet werden kann, da bald nach dem Wundverschlusse, welchen ich noch nicht für einen eigentlichen Regenerationsvorgang halte, die Neubildung des Bauchmarkes in denjenigen Segmenten, in welchen dasselbe fehlte, und zwar unter gewisser Mitwirkung des alten Bauchmarkes, stattfindet, und dass erst, nachdem diese Neubildung stattgefunden hat, die Eigentliche Regeneration, d. h. die Bildung einer Proliferationszone, vor dem Analsegment erscheint und die Entwickelung eines Regenerationskegels zustande kommt," and more poignantly "Dass Die Anwesenheit des Centralnervensytems die eigentliche Regeneration der fehlenden Kopersegmente gewissermassen bedingt, oder mit andern Worten, dass die eigentlichen Regenerationsprozesse vom Centralnervensystem auf irgendwelche Weise beeinflüsst werden."

Retzius (92) showed quite clearly that—in the earthworm—each segment is innervated by motor nerves that take their origin in its own s gment or in the next adjoining and possibly in the next two segments. On the supposition that the distribution of nerves in

<sup>&</sup>lt;sup>8</sup>Havet, J., also showed this for lumbricus and other worms in his "Structure du Système nerveaux des Annélides," La Cellule, vol. 17, 1900.

nereis is not unlike the earthworm, it is highly probable that the destruction of the nerve cord from the end, 1, 2 or 3 segments did not suffice to remove all motor stimuli from the amputated end. The point I wish to emphasize is that in order to ascertain whether the nerve cord is essential, i. e., whether it is necessary in morphogenesis, it becomes necessary to completely remove the cord from more than three segments next to the cut end. This Nusbaum appears not to have done. His proof is not complete until it can be shown that the removal of the cord for more than 3 segments either greatly retards regeneration until the fibers will have reached the end, or totally inhibits the growth of a new tail.

My experiments on the earthworm were practically completed when Nusbaum's paper was published.

I found as Morgan had found that there was a very large mortality following the operations. Furthermore, believing that the removal of tissues other than the nerve cord might introduce disturbing factors, I was led to devise some other method of removing not only the nerve cord but the bases of the lateral branches as well. The anterior 3, 4 or 5 segments were amputated. The brain and circumœsophageal commissures were removed with the anterior piece. Fine forceps were introduced into the posterior piece in such a manner that the points were close to the cord. At the desired depth the cord was seized and pulled out. It was rather surprising to find how readily the cord and considerable pieces of the lateral nerves could be removed. The piece was examined under the microscope and the number of ganglia counted. Fig. 14 is an example of a nerve cord that was extracted in this way. Sometimes the nephridia were extracted instead, but a glance through the microscope revealed the error and the particular worm was discarded. In every worm to be hereafter mentioned, Table 4, the "brain" and circumœsophageal commissure, and the whole of the cord for a given number of segments, together with the basal parts of their lateral nerves were removed. The amputated end of such worms was therefore intact except for the absence of the cord in from 2 to 11 anterior segments.

Similar operations were made at the posterior ends of pieces cut near the middle or posterior levels of the worm. But the injured

segments sloughed off, either at the time of operating or after several days. Owing to this autotomy the regeneration of the new tail began not from the cordless level but from the more anterior regions, where the organs were uninjured. In every case examined, the injured, and often many of the uninjured segments as well, had been cast off. In No. 3.6 for example the piece contained 85 segments. The cord had been removed from the posterior 8 segments. At the time of fixation however there were but 73 segments. In this animal 8 injured as well as 4 uninjured



Fig. 14 Nerve cord of the earthworm, extracted according to method described on page 693. Drawn from fresh specimen.

segments had sloughed off. No further work on posterior level was carried on. Autotomy near the anterior end of the worm was very rare and every such case was discarded. The 5th segment with its reproductive opening offered a very convenient point from which the number of segments could be counted.

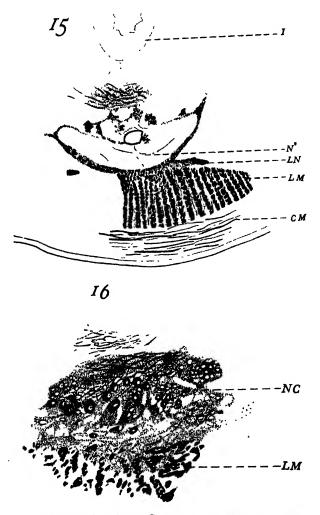
The earthworms were isolated, in separated flower pots, containing damp earth. They were placed on top of the earth and remained there for 25 to 40 days. When stimulated they reacted sluggishly. Beginning about 25 days after the operation, some

worms began to disappear below the surface. Examination showed that in every case a head with a functional mouth had been regenerated. About 250 worms were operated upon. The mortality was low; in some series only from I to 5 per cent died. Of the survivors about one-half regenerated heads. It should be borne in mind that in order to obtain the early stages in the changes of the injured regions, a large number of worms had to be preserved long before it could be determined whether a head would or would not regenerate.

According to Retzius the nervous system in the earthworm comprises two quite dissociated parts. The motor cells are found exclusively in the cord, the sensory cells in the skin. The cord cells are of two general types; the great majority are unipolar cells the processes of which soon bifurcate, the branches passing into the next anterior or posterior segment or to the other side of the same segment. There is a second type of cells usually very much larger, with its branching neurones passing in all directions but rarely beyond the limits of the segment. It is thus seen that the motor nerves in any segment take their origin in the same segment or in the next anterior or posterior one. The sensory cells send their nerve fibers into that part of the cord within the same segment. order to remove from an amputated end all the motor stimuli the cord must be completely removed from at least 3 segments adjoining the cut surface. Concerning the giant fibers, so called, and their functions, practically nothing is known. Even their nervous nature is questioned. They are believed (Clarapede) to take their origin near the anterior end of the nerve cord. As these "fibers" are intimately bound up with the cord, they were removd with the rest of the cord from the amputated end.

Table 4 gives a list of individuals taken from one series (C); the number of segments amputated, the length of cord removed, the time, the number of segments regenerated, etc., are also given. The rate of regeneration was extremely variable, and apparently independent of the number of segments amputated and the length of cord removed. There were of course other injuries particularly to the mesenteries, nephridia and sometimes the inner muscle layer. It is possible that the rate of regeneration was largely

determined by the extent of these injuries. It is certain that the operated animals required more time to produce a head than the control animals.



Figs. 15 to 16 Cross-sections through ventr# portion of the earthworm 12 days after operation. Fig. 15 shows absence of nerve cord and proximal part of lateral nerves. Fig. 16 (section through 7th segment, i. e., through anterior end of nerve cord) shows presence of a few ganglion cells scattered among large numbers of smaller cells. CM, circular muscles; LM, longitudinal muscles; LN, proximal end of lateral nerve; N, space formerly occupied by nerve cord; NG, nerve cord; S, sheath of cord; I, intestine.

### Head not Regenerated

As none of the worms were amputated posterior to the 5th segment, the lack of regeneration could not have been due to the fact that the level had been reached beyond which no anterior regeneration takes place. Nor was it due to length of cord removed, for in other cases as much or more of the cord was extracted, yet a head appeared.

Histologic examinations of these worms at different periods gave the following results:

No. 1.79. The 3 head segments were removed. The ventral



Fig. 17. Cross section through 8th segment, i. e., through old cord. Sheath is present. Ganglion cells more numerous and arranged around periphery. Letters as in Figs. 15 and 16.

nerve cord was extracted from the next 3 segments. Preserved 12 days later.

Segments 4 to 6' inclusive contain no trac of the cord or bases of the lateral branches. Fig. 15 is a cross-section through the 6th segment showing the ventral portions of the worm and the complete absence of the cord. Toward the 7th segment the ventral

<sup>&</sup>lt;sup>7</sup> The original number of the segment will be used, thus, if 3 segments were removed and none regenerated, then the first segment of the headless animal is the original fourth segment and will be so designated.

space in the anterior segments is filled with a loose mass of tissue, partly degenerating muscles, partly connective tissue in which are many blood cells and other small cells. Among these small cells (primitive nerve cord cells) are a few ganglion cells, Fig. 16. These latter are round or pear shaped, with relatively enormous nuclei, and with a distinct nucleolus. (Fig. 16 represents a greater magnification than Fig. 15.) A little farther posteriorly the number of typical ganglion cells increases, and these are arranged around the periphery. There is also present the membrane surrounding the cord, Fig. 17 (same magnification as 16). In the 8th segment the cord is quite normal.

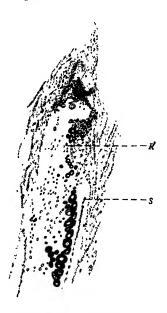


Fig. 18 Longitudinal section of early stage in regenerated cord. Migration of ganglion cells and collection of masses of small cells clearly shown. S, sheath of cord; R, regenerated cord.

No. 1.94. Amputated anterior 4 segments. Removed cord from the next 4 segments. Time, 20 days. Segments 5 to 8, inclusive, contained no cord. A cap of blood cells and minute nerve cells had formed around the anterior end of the cord.

In 1.47 the cord was removed from 2 segments. A longitudinal section, Fig. 18, is instructive. The broken end of the old cord is marked by the termination of the cord sheath, as well as by the absence of the typical ganglionic cells. These have divided into smaller ones by active division. The minute cells also appear to have multiplied rapidly and collected at the anterior end. Many stages in the development from these minute cells, almost to the typical ganglion cells, may be seen in Fig. 18. Another point of particular interest is the forward migration of this whole mass of cells.

In 1.8 the cord was extracted from 5 segments. Time 68 days. Though the time was twice as great as the preceding, constructive changes have gone no farther.

The forward growth of the cord is even more pronounced in No. 1.16. From the anterior end of the old cord, nerve cells of all sizes and all stages of differentiation, from minute cells, difficult to distinguish from surrounding cells, to typical ganglion cells have pushed forward to the amputated end, yet no regeneration of a head was induced. These changes required 82 days for their completion.

The worms next to be considered have regenerated even a greater length of cord than any of the preceding.

No. 1.107. Cord extracted from 3 segments. Time, 28 days. From the end of the old cord at the 6th segment, the new cord has grown anteriorly about one segment. It tapers and loses itself among a mass of fibers and connective tissue. The new cord contains cellular and fibrous layers, though very primitive.

Though No. 1.7 was preserved 61 days, after the operation, the characters are essentially the same as 1.107.

In No. 1.4 after 79 days the new cord has grown a trifle over 1 segment in length.

No. 1.33. Cut off 3 segments. Extracted cord from 4 segments. Time, 35 days. The old cord is normal until the 9th segment. From this level the cord quickly shrinks, owing largely to the diminution of the fibrous layer. Beginning at the middle of the 7th segment the new cord has grown into the 6th and 5th segments. Along the new cord are very many minute nerve cells that cluster into primitive ganglionic masses in the middle of the 6th and 5th segments. At these places the cells assume more of the typical appearance of the ganglion cell.

No. 1.40. Three segments removed. Cord removed from 3

segments. Time, 35 days. The cord has grown still farther forward from the 8th to the beginning of the 5th segment. Even more clearly than in the preceding, the disappearance of the large nerve cells near the broken end of the cord and the forward growth of nerve cells and fibers are seen. The nerve cells collect in each segment to constitute primitive ganglia, Fig. 19. Anteriorly the cord ends in a mass of connective-tissue fibers. In none of the

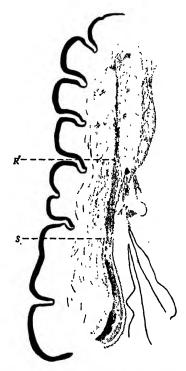


Fig. 19 Longitudinal section. Later stage in regeneration of nerve cord. Cord has grown from old cord anteriorly 32 segments. No evidence of contribution from ectoderm. Head not regenerated.

preceding cases is there any evidence of cord formation from the amputated end. The growth took place entirely from the old nerve cord.

No. 1.1. Amputated 3 segments, extracted the cord from the next 5 segments. Time, 50 days.

Regeneration of the new cord anteriorly has progressed even

farther than 1.40, or, more correctly, the elementary cord has assumed more of the characters of the original cord. From the 15th to the 9th segment, the cord is normal. From here to the 8th segment it tapers, due to the degeneration of the fibrous layer. The attenuated cord reaches almost to the 4th segment. The new cord is considerably wider than in 1.40, the cluster of cells in each segment approach more closely the typical ganglia, and the constituent cells resemble more and more the ganglia cells of the normal cord.

It has been pointed out that the removal of the cord from the two segments nearest the cut end sufficed to prevent any motor nerve stimuli from reaching that end. In the preceding experiments, the whole cord, together with the bases of the lateral branches, was removed from the 3d, 4th and 5th anterior segments. The length of the extracted cord was made doubly certain, first by examination under the microscope immediately after the operation, secondly by subsequent study by serial section. In none of the instances given was there any sign of the regeneration of the head, though the time in some cases at least was more than three times what is ordinarily required for a head to regenerate.

Of the early changes near the broken end of the cord three deserve special consideration:

- I Degeneration of the fibrous layer of the cord does not extend over one segment from the broken end.
  - 2 A cap of small cells develops at the broken end of the cord.
- 3 The large nerve cells disappear and are replaced by large numbers of smaller cells.

Subsequently the nerve fibers in the cord grew forward to and beyond the broken end, pushed aside the cap of cells and carried forward the numerous small nerve cells. These tended to form clusters of primitive ganglionic masses in the middle of each segment. In the latest stages this anterior growth had extended over several segments, to within one segment of the amputated end. There is no evidence whatsoever of growth of the cord from the ectoderm.

The question arises whether the lack of regeneration of the head may not have been due to the failure of the nerve cord to grow forward to the amputated end. In other words, where the nerve cord has not yet succeeded in reaching the cut end no regeneration of the head occurred. The following results negative such a conclusion for they demonstrate that a head can be regenerated in the entire absence of the nerve cord.

## Head Regenerated-New Brain and Old Cord Not Connected

The new head, made up of two or three segments, did not differ externally from those to be considered in the next section.

No. 1.29. Rem ved 3 segments, 5 ganglia of the cord extracted. Examined 35 days later.

The old cord extends anteriorly as far as the middle of the oth segment. The regenerated cord has grown from this level through the 8th and almost to the anterior nd of the 7th segment. The changes in the cord both old and new are exactly like those described in the preceding section. Segments 6, 5 and 4 contain not the slightest trace of a cord or nerve cell or nerve fibers, nevertheless a head has formed, the number of segments of which are not easily made out; Fig. 20. In the head is a well-formed "brain" -not quite separated from the ectoderm from which it has arisen (not clearly seen in the figure). The ectoderm, as already described by other investigators, has proliferated in two directions, dorsally and posteriorly to form the cerebral ganglia, ventrally and posteriorly to constitute the commissures. In this particular animal these commissures have not grown very far, though enough to indicate their character and perhaps to foretell what would have occurred if the animal had been allowed to live.

There can be no question but that the new functional head had developed entirely independent of the old nerve cord.

No. 1.19. Amputated 3 segments. Removed 7 ganglia. Time, 69 days. This is substantially the same as the preceding. The old cord reaches to the middle of the 10th segment. The new cord has grown forward nto part of the 8th segment. The anterior end of the animal, including segments 4 to 7½ inclusive, does not have the faintest trace of any part of the cord. The new head like the preceding contains cerebral ganglia and com-

TABLE 4.

Series C. Earthworms that regenerated a head, in which the cephalic ganglia are either continuous with old cord or separated from it by cordless segments.

NO.	SEGMENTS REMOVED	NERVE CORD EXTRACTED FROM	SEGMENTS REGENERATED	BRAIN REGENERATED	RRAIN AND NERVE CORD CONNECTED	TIME
		aegments	i			daye
1.1	3	11	0	no	no	50
1.4	3	5	0	no	no	79
1.7	3	4	•	no	no	61
1.8	3	5	•	по	no	68
1.9	4	3	0	100	no	30
1.11	3	9			1	29
1.12	3	5	2	yes	yes	85
1.15	3	9	2	yes	no	36
1.16	3	}	•	no	no	82
1.17	3	7	3	yes	yes	64
1.19	3	8	2	yes	no	69
1.22	3	6	2	yes	yes	92
1.26	3	3	2			44
1.27	3	23	+	yes	yes	35
1.29	3	5	1	yes	no	35
1.30	3	2	3	yes	yes	35
1.31	3	3	τ	yes	yes	35
1.33	3	2	0	no	ю	35
1.34	3	1	3	yes	yes	38
1.35	3	1	3	yes	yes	38
1.37	3	33	2	yes	yes	35
1.38	3	3	3			40
1.39	3	3	2			40
1.40	3	3	•	no	no	35
1.41	3	2	4			42
1.43	4	3	3	yes	no	46
1.44	4	3	2	yes	yes	43
1.45	4	4	2	yes	yes	43
1.46	4	2	2			46
1.47	4	1	0	no	no	34
1.48	4	3	3	yes	yes	43
1.49	4	2	3			46
1.50	4	3	3	yes	yes	43
1.54	4	5	2	yes	yes	43
1.55	4	5	3	yes	yes	46
1.58	4	2	3			50
1.59	4	4	2	yes	yes	43
1.60	4	4	3			47

TABLE 4 (continued)

NO.	SEGMENTS REMOVED	NERVE CORD EXTRACTED FROM	SEGMENTS	BRAIN REGENERATED	BRAIN AND NERVE CORD CONNECTED	TIME
		segments				days
1.61	5	3	2 ,	yes	yes	43
1.65	5	3	•	no	no	55
1.66	5	3	0	no	no	44
1.68	5	2	· 4 .	i		43
1.78	3	4	. • !	no	no	7
1.79	3	4	•	no	no	12
1.89	3	3	, o '	no	no	16
1.94	4	: 5	, • 1	no	no	20
1.96	3	2	}	yes	yes	22
1.97	3	4	2			24
1.98	3	4	, • ;	no	ро	24
1.102	3 '	-3	' • ;	ļ		26
1.103	3	2		!		26
1.106 etc.	3	21/2			1	28

missures, but the latter have grown farther ventrally, where they meet to form the first subæsophageal ganglion.

No. 1.15. Amputated 3 segments. Removed cord from 9 segments. Time, 36 days. This animal is interesting not only because a new functional head has developed without any connection with the nerve cord, but because the distance between the cord and the new head is over 8 segments, a distance far too great for any nerve impulse from the cord to reach the amputated end. The "brain" is connected by commissures which, however, end about one-half way towards the ventral side. Segments 4 to 11½ inclusive have no trace of the cord. At the posterior end of the 12th segment, the early stages in the regeneration of the cord are observed, which in the 13th segment are replaced by a typical cord.

### Head Regenerated-New Brain and Cord Continuous

The results may be arranged into two groups, in the early stages at least, according as the cord has grown more rapidly towards the head, or regeneration from the ectoderm has been more rapid in the reverse direction. The cord appears in some cases to have met midway between the old cord and the amputated end. Later

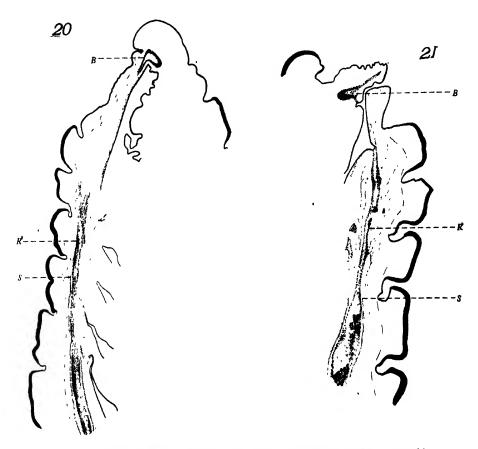


Fig. 20 Longitudinal section. Shows (1) anterior growth of the new from the old nerve cord; (2) development of cerebral ganglia and commissures in new head; (3) absence of nervous tissue in intermediate segments.

Fig. 21 Longitudinal section. Cerebral ganglia in regenerated head connected with the old nerve cord. It is very probable that new cord has grown posteriorly as well as anteriorly. B, regenerated cerebral ganglia; R,' regenerated nerve cord; S, sheath surrounding old cord only.

changes completely obscure the early steps in the formation of a continuous cord.

In the following individuals the regenerated cords are very much

like those described in the first section. But instead of ending blindly as the latter do, they swerve dorsally, near the amputated end, become filled with nerve cells to the almost complete obliteration of the fibrous layer, and end in the cerebral ganglia.

In No. 1.59 for example, the greatest width of the cord measured in the middle of each segment is as follows:

SEGMENT	Units <sup>8</sup>	
5th	5 }	
6th	5	
7th	5	
8th	5 rege	nerated cord.
9th	5	
roth	5	
11th	10	
12th	II old	cord.
etc.	,	

At this last level the cord widens a trifle and merges into the "brain."
No. 1.31 is practically the same.

SEGMENT	UNITS	
4th	5 ]	
5th	5	
6th	5	regenerated cord
7th	5	
8th	10	-141
9th	13	old cord.

In the following three worms posterior growth seems to have taken place with great vigor, so that the nerve cord immediately behind the cerebral ganglia, and ventral to it, is crowded with nerve cells in all stages of different ation. The cord tapers about midway between the old cord and the new "brain," widening towards the brain on the one hand and towards the old cord on the other.

In 1.54 the cerebral ganglia have not yet separated from the ectoderm from which they take their origin. In 1.48 and 1.27 the separation is completed. Fig. 21 is a longitudinal section of 1.27 showing the ventral and anterior end of the worm. In the 8th segment the cord is quite typical containing large ganglion cells

<sup>&</sup>lt;sup>8</sup> Measured with eye-piece micrometer, with the same magnification in each case. These relative measurements suffice for the present purpose.

with their axis at right angles to the axis of the cord. In the 7th segment these have divided repeatedly to form a mass of smaller cells, with their axes at a sharp angle to the axis of the cord. In the anterior end of segment 7 and in the 6th, there is a steady diminution in the width of the cord and an absence of large ganglion cells. In the 5th segment the cord increases in diameter, in the total number of nerve cells and in the number of larger nerve cells. These are not well shown in Fig. 21 nor is the greater width of the cord in the 4th segment shown in this figure. It appears highly probable that the more extensive proliferation of nerve cells and their fibers from the ectoderm posteriorly has met the forward growth of the cord near the posterior end of segment 5.

No. of animal	1.54	1.48	1.27
Greatest width of cord in each segment	reg. cord   5   3   3	reg. cord   5   3   5	reg. cord 6 5
Greatest width of cord in each segment	old cord 10	old cord 10	old cord 12 13

No. 1.61 and 1.44 are worth mentioning. Only two and three ganglia respectively had been removed and subsequent regeneration of the cord took place in such a manner that the greatest width in each segment, beginning at the anterior end was 8, 6, 11, 12, 13, 13, etc., units and 9, 5½, 10, 10, 12, 12, etc., units respectively. In other words the width decreases from the commissures posteriorly. The ganglionic masses seem also to be more highly developed at the anterior segment, less so in the next segment. This evidence seems to strengthen the conclusion that from the anterior end, the new cord had regenerated posteriorly as far as the growing end of the posterior cord.

The growth of the new cord posteriorly may be relatively faster than the forward growth from the broken end of the cord. At the anterior end, the nerve cord resembles more and more closely the typical cord, both in dimensions and differentiation of the cellular constituents, while the posterior end of the cord is far less typical. The regenerated cord thus tapers posteriorly.

NO. OF SEGMENT	4th	5th	6th	7th	8th	9th
No. 1.43	ı	9 14 11	4 10 <del>1</del> 5	4* 10* 5*	7–10 6–10 12	13 13 12 etc. units.

<sup>\*</sup>Level at which old cord begins.

In Nos. 1.30, 1.55, 1.17 and 1.22, no clear separation between the two parts of the regenerated cords could be made, and in some of the animals no sign of former injury was discernible.

#### Summary

We have shown that considerable lengths of the cord may be completely removed without causing the death of the animal.

The cord so removed has attached to it the base of the lateral nerves, that also contain nerve cells. The rest of the lateral nerves have no cells.

The removal of the cord from three or more segments completely shuts off from the amputated end any motor impulses and thus we are enabled to ascertain whether regeneration of a head is dependent on the presence of motor stimuli.

About 50 per cent of the operated individuals regenerated a head (see Table 4).

The head regenerates rather later in these operated animals than in the control animals.

The worms that did not regenerate a head showed unmistakable evidence of anterior growth of the nerve cord, that extended over several segments, and in some instances almost to the amputated level.

At first the broken end of the cord was covered by a cap of small cells. The large ganglion cells near this end disappeared and were replaced by numerous minute nerve cells that migrated forward. These congregated into masses to form the primitive ganglia. The fibrous layer also tended to increase in thickness.

The presence of this regenerated cord quite close to the amputated end did not suffice to induce development of a head.

Where a new head did appear the cerebral gang'ia proliferated from an invagination of ectoderm in two directions, (1) dorsally to form ganglia proper, (2) ventrally to form the commissures.

These new and functional heads with their cerebral ganglia may develop in the entire absence of motor nerve influences. In the three animals where this clearly occurred, the old cord was removed from 3½, 4½ and 9 segments respectively, and though some anterior regeneration of the cord took place, the intervening area between this and the amputated end did not contain the slightest trace of any part of the cord.

In the great majority of worms with regenerated heads, the brain was connected with a continuous cord, which arose very probably by the anterior growth of the cord on the one hand and the posterior growth from the anterior "proliferation zone" on the other.

Here we have a second instance where an adult animal regenerated an organ without the aid of the nerve cord, or of motor nerve stimuli.

# REGENERATION IN THE STARFISH IN RELATION TO THE NERVOUS SYSTEM

A few experiments were undertaken with the common starfish, Asterias vu'garis, to ascertain whether the presence of the nervous system is requisite for regeneration. The nervous system includes, in the arms at least, (I) a nerve ridge of the apical nervous system situated along the roof of the coelomic cavity, (2) radial nerve ridge of the superficial oral system, (3) ridges of the deeper oral system. King believed that only the oral nervous system was needed in regeneration. For, the oral half regenerated the aboral half of the arm, while the reverse was not true. But the inability of the aboral to replace the oral half may have been due to the absence of other systems essential to the formation of the arm. For with the oral half there were removed the water vascular, the hæmal system as well as the oral nerve ridges. Experiments

on Antedon (Przibram '01) gave quite the contrary results. After the removal of the oral disc with its nerve ridges, the missing parts were regenerated perfectly, but when the aboral system within the central capsule was injured or destroyed, regeneration was inhibited. My own experiments consisted in cutting wedgeshaped pieces from the amputated ends, either on the oral or aboral surfaces or on the side, different arms being used for each of these operations. With the aboral piece a portion of the apical nerve together with adjacent tissues was removed; the oral piece contained the two parts of the oral nervous system together with part of the ambulacral system and other tissues; the lateral piece did not include any of the nerve tracts. In the first two instances regeneration of the arm was inhibited until the space had been almost filled in by new tissue, after which regeneration of the rest of the arm took place. The time required for the aboral surface to complete itself was very brief, for the oral surface, considerably longer. These facts may be interpreted to mean that both the oral and the aboral nerve ridge must be present at the amputated surfaces before regeneration begins. The absence of either one prevents for a time at least the formation of a new arm. That this is not the correct interpretation is shown by removal of nerve ridges only, without seriously injuring the other tissues, and by leaving the amputated surface otherwise complete. This was done in a group of 80 small starfish from each of which several arms were amputated at the same level. By means of a broach or a hot needle the apical nerve ridge of at least one arm and the oral ridges of another were destroyed. The latter method was found to be worthless for arms so treated invariably disintegrated to a more proximal level. The broach method was more satisfactory. The rough treatment often caused the arm to break off at its base, or to slough off the injured part. Such animals were discarded. In some, the saw-like action of the broche divided the oral surface in two, thereby destroying both layers of the oral system. Whatever the mode of operation the result was the same, a new arm was regenerated.

In about 15 days the eye spot was discernible in the operated arms and in the non-operated arms. In many instances to be

sure the eye spot of operated arms appeared several days later. The development of new ambulacral feet and the skeleton of the new arm took place in both the control and nerveless arms. The rate was about the same although some of the operated ones lagged behind. Further development was approximately the same in both.

# REGENERATION IN PLANARIA IN RELATION TO THE NERVOUS SYSTEM

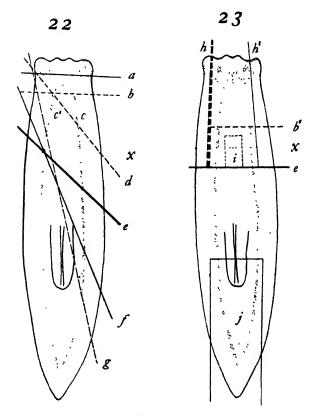
The investigations of T. H. Morgan, Child, Lillie, Stevens, L. V. Morgan, Shultze and others have shown that different species and genera of planaria differ radically in the power to replace lost parts. To cite but a few examples. Planaria maculata is able to regenerate a whole worm from a piece taken from almost any level; Dendrocœlum lacteum like P. maculata can regenerate posteriorly at all levels but anterior restoration takes place only in that third of the animal nearest the head; in Leptoplana anterior regeneration is still further restricted to the region immediately back of the cephalic ganglia. These facts suggested some connection between the reduced power of regeneration and the increased cephalizat on of the animal Lillie advocated this view after an examination of the nervous systems in three different planarians.

L. V. Morgan and Bardeen believed that whenever the anterior and of the nerve cord was exposed a head regenerated at that place. Child made the interesting discovery that the formation of a head could be prevented by removal of the "brain," and that the extent of the regeneration was in proportion to the amount so removed.

More recently Child has inclined to the view that "the relation between the nervous system and morphogenesis is of a very problematic character, though the existence of a relation of some sort can scarcely be denied in many cases. This relation may be either direct or indirect." He states his position as follows: "A change in kind or degree of functional activity is of course accompanied by changes in the complex of functional conditions to which the part is subjected. If these conditions play any part in the morphogenesis or form maintenance a relation between the nervous system

and form will appear to exist in such a case, but upon analysis will be found to be indirect rather than direct."

If the former views of Child and of Lillie and Morgan are correct we must suppose that the removal of the cephalic ganglia alone (Child—Morgan, L. V.) suffice to inhibit anterior regeneration in



Figs. 22 and 23 Planaria, Dendroceslum lacteum, indicating the different kinds of operations described in text.

Leptoplana, while these ganglia together with the anterior third of the cords must be removed in Dendrocælum to bring about the same results, but in Planaria the nervous system is so diffuse that removal of the whole of the central nervous system cannot prevent regeneration.

The following experiments that I made on Dendrocœlum lacteum may throw some light on this question:

Experiment 1. Section at level a of Fig. 22 leaves the anterior piece presumably without cephalic ganglia. Such pieces died within a few ays.

Experiment 2. Section through the cephalic ganglia. The anterior piece usually dies in a few days. Survivors do not regenerate posteriorly. In one such piece a heteromorphic head was regenerated.

Experiment 3. Section through b or any level anterior to X. Posterior pieces regenerate a head, anterior pieces a tail. If a cut be made through any level posterior to X the anterior piece regenerates a tail, but the posterior piece does not regenerate a head. X is the imaginary line that marks the level posterior to which no head is regenerated. In one case however the posterior piece cut just beyond the anterior end of the pharynx regenerated a heteromorphic tail. On the view previously mentioned, the absence of cephalic ganglia and that portion of the two cords anterior to X and marked c' and c, is responsible for the lack of formation of the head. This view was tested by the following experiments.

Experiment 4. The animals were cut along the line d, removing ganglia and a part of the cord c, anterior to X. Both pieces complete themselves to form two typical animals.

Experiment 5. If d vided at e, the posterior piece contains a small portion of the region anterior to X, but without the cords c or c', or the ganglia. No anterior regeneration took place.

Experiment 6. If the cut be made through f or g the posterior piece like the preceding contains neither ganglia nor cord c and c'.

It contains more tissue anterior to X. Under these circumstances the projecting piece often sloughs off or shrinks down to a more posterior level and, like those planaria in Experiment 5, produced no head. When little or no sloughing takes place a head is regenerated, which may be complete and ultimately typical, or incomplete. It would appear as though more incomplete heads tended to appear on the longer strips, though I cannot be certain about this.

Experiment 7. When a square piece, b'h, Fig. 23, including the ganglia and a large part of c and c' is cut out, regeneration of a head takes place as we should expect. When eh is removed, so that e occurs posterior to X and the strip h contains neither ganglia nor c and c', a head is regenerated, which may be either perfect or imperfect. The head grows in different places depending on the nature of the original injury, and on the extent of the subsequent shrinkage or sloughing. If the latter takes place the resulting individual regenerates a head when the level is anterior to X, an imperfect head when close to x, and no head posterior to X. If the strip h is long the new head may form altogether from the cut side, without any apparent contribution from the posterior level as at d, in Fig. 22. In other words when a piece of sufficient bulk anterior to X, but not containing ganglia nor cords c and c', is left attached to the animal, such a piece can regenerate a new head. It appears to be a question not of nerve supply but of mass or kind of material.

Experiment 8. This experiment is interesting because it shows that still another factor has to be reckoned with, in anterior regeneration. If we remove the middle area including ganglia - and cords, and leave two narrow bands h and h', Fig. 23, these come together, curl upon one another and may behave in any one of the following ways: The strips may break off at their bases, b' or e; if near the former level a new head is produced, if near the latter, no regeneration takes place. Where little or no sloughing occurs, lateral regeneration between the cut strips may take place and a new head is regenerated. Very often however particularly where the strips are long, i. e., they extend back of x, the two strips embrace each other firmly, and no lateral regeneration occurs. Such animals lived for a long time but no head was regenerated. It was shown in a previous experiment that each of these strips has latent the power to regenerate laterally, yet here something prevents the piece from exercising its power. For lack of a better term. I have called this inhibiting influence the mechanical interference of parts. Stevens has found a similar phenomenon in other planarians.

Experiment 9. A narrow strip i, Fig. 23, was left projecting

anterior to x. The strip was without ganglia or cords. When no shrinkage took place a new head regenerated. This is in accord with conclusions of Experiment 7.

Experiment 10. By cutting a long V-shaped piece from the anterior end, the cephalic ganglia and large parts of the cords c and c' are removed, and a larger portion of the body of the animal is left anterior to X. There is great variation in the result. If both strips are nearly equally long the animal behaves like those in Experiment 8. If one is considerably longer than the other, the resulting behavior is like Experiment 7. If both ends slough off, no regeneration occurs.

Experiment 11. When a  $\Lambda$ -shaped piece was cut from the head end, at such a level that the ganglia and major part of cords c and c' were removed, a new head was invariably formed. Both cut sides of the  $\Lambda$  contributed to the formation of the new head.

Experiment 12. Each longitudinal half of the animal can replace the missing half, and thus produce two whole organisms. Even when the animal is cut into two unequal parts regeneration takes place in each, provided the smaller piece is not too small. The exact size below which no regeneration occurs is difficult to determine. It is certain however that many such pieces containing nearly the whole of one of the nerve cords never regenerate.

Experiment 13. The animals were cut longitudinally into two nearly equal parts and then each half was cut across, either anterior to X or posterior to it. More frequently the operations were made in the reverse order. The anterior pieces developed into complete animals. The posterior parts also developed into complete animals provided the cross cut was anterior to X. In other words, lateral as well as posterior and anterior regeneration took place. When however the cross cut was made posterior to X, all regeneration even the lateral regeneration was inhibited.

Experiment 14. The result in the previous experiment stands in marked contrast to that obtained after first dividing the animal at any level posterior to X, and then cutting the tail across. A new tail regenerates. This was pointed out by L. V. Morgan in Leptoplana.

According to the prevailing view we should believe that the

removal of the cerebral ganglia and that part of the cords called c and c' inhibits anterior but not posterior regeneration. Furthermore, removal of the cerebral ganglia and the cord on one side, or the removal of the ganglia and the cord anterior to X, Experiment 13, does not prevent lateral regeneration. Yet if I mm. or perhaps a fraction of a millimeter more of the cord be removed, lateral growth is prevented.

Experiment 15. The mechanical interference of parts mentioned in Experiment 8 is also shown, where one might least expect to find it, viz: in the posterior part of the body. A long central strip j, including both cords, was removed from the tail (Fig. 23). The two remaining flaps came together. If these sloughed off regeneration took place from the more anterior end. When sloughing did not take place the two strips remained firmly united, preventing in the great majority of the planaria any lateral regeneration of tissue between the strips.

# Summary

It is improbable that the removal of the nervous system in the anterior third of the planarian D. lacteum is responsible for the lack of regeneration of a head in posterior pieces, because,

- I The removal of this part of the nervous system does not prevent regeneration of a tail;
- 2 The presence of only one-half of this portion of the nervous system suffices to permit regeneration of a head and lateral half of the animal;
- 3 It was further shown that a fraction of one of the cords suffices for the regeneration of a head;
- 4 Finally, regeneration of a head took place when the whole of the cerebral ganglion and cords c and c' were cut away.

The essential condition in the above categories seems to be the presence of a sufficient quantity of body tissues anterior to X.

The latent power to replace missing parts may be checked by means other than the removal of the nervous system, namely, by the "mechanical interference of the parts." In this way both anterior and posterior regeneration can be prevented. (Experiments 8, 10, 15.)

These experiments together with those on earthworms and newts should make one cautious about accepting the view of the direct or even indirect influence of a nervous influence on regeneration.

### CONCLUSION

Since summaries of each of the foregoing sections have already been given, I wish here only to emphasize certain essential points.

No pains were spared to establish beyond dispute that the nervous system had been completely removed in those animals used to test this question. In no work heretofore published has this important consideration received sufficient attention. Wolff's experiments most closely fulfill the conditions, yet in many of his animals the legs moved after three months, clearly indicating that all connections had not been destroyed or that new connections had been established.

In the newt and in the earthworm at least, and very probably in the tadpole of the frog as well, all sources of nerve stimuli to the amputated region were destroyed. In these animals, the usual physiological tests to determine reflex action were supplemented by histological examination of the regenerated and adjacent regions. In this way the exact nature of the initial injury to the nerve cells and their fibers, and the consequent destructive and constructive changes of the nerve as well as the adjacent tissues were exactly determined in over one hundred different animals.

In the hind leg of Diemyctylus for example, it was found that the nerve cord had been totally destroyed in the region of the lumbosacral plexus, in the tail and in one to six vertebræ anterior to the plexus. The destruction of the cord for so great a distance on either side of the plexus renders it certain that no motor stimuli could have reached the hind legs either from anterior or posterior levels. In many of these and in other animals, the sensory or spinal ganglia of the plexus and other nerves were also destroyed. Examination disclosed the fact that no subsequent regeneration of the nerve cord or ganglia or of the corresponding motor and sensory fibers took place within nine months after the operation. The legs were completely paralyzed during the whole of this time. Despite the lack of all nerve connections, the typical structure of the leg, foot and toes were developed in every case.

After removal of nerve stimuli the regenerated organs may show certain malformations, but these are traceable to indirect effects of the operation upon nutrition, respiration, etc., of the tissues affected, or to mechanical stresses. The primary fact of a normal regenerative process resulting in the development of all the parts typical of the missing organ is unquestioned.

In the case of the tadpole, the evidence is not so conclusive. For, in order to prevent all nerve stimuli from reaching the amputated end, it is necessary to destroy the nerve cord and the sensory ganglia from practically the whole of the tail. This was difficult to do. I succeeded however in destroying the nerve cord in the tail except for isolated and very much injured fragments whose nerve connections appeared to have been completely broken. In this way all or very nearly all motor and sensory nerve stimuli were prevented from reaching the amputated end. A new tail however regenerated in every instance.

The evidence is equally clear that even when motor and sensory stimuli reach the amputated region, regeneration cannot take place, if certain other organs are absent. For example, the formation of a new tail in the frog tadpole may be completely prevented by removing the notochord from the cut end. The nerve stimuli from the intact nerve cord and sensory ganglia are not able to start the regenerative processes. In Diemyctylus also, development of a new tail can be prevented either by removing the skeletal axis of bone or cartilage, as in the tadpole, or by preventing the nerve cord from reaching the amputated surface. By the second method nerve stimuli are prevented from reaching the cut end by extracting a sufficiently long piece of the cord; or, by merely placing a very small plug at the end of the vertebral canal, the cord is prevented from reaching the amputated end without thereby interfering with the innervation. Tails so treated did not regene-In other words the absence of the skeletal axis or of the nerve cord from the end of the tail stops regeneration completely, although nerve stimuli were present. No one speaks of a "morphogenic" influence exerted by the bone or cartilage. Have we any more reason for referring such an influence to the nerve cord?

The ability of adult animals to regenerate in the total absence of innervation from the central nerve system is not limited to verte-

brates, for in other forms as well, differing widely in organization such as the earthworm, the starfish and the planarian, the same result was obtained.

The earthworm affords a clear case. Study of serial sections established beyond all doubt that the whole of the ventral nerve cord and the cellular part of the lateral nerves had been removed from the region comprising from 2 to 9 segments nearest the amputated anterior end. Out of 200 operated worms approximately one-half regenerated a functional and typical head. On examination it was found that a new nerve cord had regenerated between the old cord and the new "brain;" and it appeared that this regenerated cord grew rapidly from the old cord anteriorly and partly from the anterior end in a posterior direction. In three worms at least, the regeneration of the cord had proceeded so slowly that the "brain" of the regenerated head and the anterior end of the old cord was separated by a space of three to eight segments, entirely devoid of any trace of the nerve cord or of connecting motor fibers. This distance is far too great to permit any secondary nerve connections. We are thus led to conclude that the head of the earthworm may regenerate entirely independently of the nerve cord.

On account of the diffuse character of the nervous system in the starfish and in the planarian, the absolute removal of all nerve cells from the amputated region is rendered next to impossible. The experimental evidence, however, showed that even after removal of the principal nerve tracts of both starfish and planarian, regeneration takes place quite readily.

These results are in full harmony with the recent investigations of Schaper, Rubin, Barfurth, Harrison and others, viz: that larval development is independent of a nervous control. They are also in entire accord with the more recent results according to which functions formerly attributed to the central nervous system are now relegated to other agencies.

We reach, therefore, the general conclusion that regeneration of typical organs in adult as well as in larval animals can take place in the entire and permanent absence of any influences exerted by or through the central nervous system.

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## ATAVISM IN GUINEA-CHICKEN HYBRIDS

BY

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(WITH FOUR PLATES)

In 1905 the writer came into possession of five adult hybrids which had been produced by crossing a cock of the black langshan breed of chickens with an ordinary domestic guinea-hen. The present paper is concerned chiefly with a curious color pattern which manifested itself in these fowls. It is unlike anything in the coloration of either parent and is to all appearances atavistic in nature.

Concerning the immediate ancestry of the five hybrids, little need be said as both are common domestic fowls.

The guinea (Fig. 1, Plate I) is of clumsy build and weighs only about three and one-half pounds. It is remarkable for the yellowish or brownish bony helmet which surmounts its naked head. Its neck is also naked except for a few bristle-like feathers which form a median row up the back and which may also in some individuals be sparsely scattered along the sides and front. A median longitudinal fold of skin under the throat is more or less developed in most individuals. Broad, red gape-wattles are present and the cere and base of the beak are of reddish hue. The sides of the face and upper neck are white with a decidedly bluish tinge. The top of the beak is of orange color shading to light horn towards the tip which curves sharply downward. The neck, very small just

<sup>&</sup>lt;sup>1</sup> I wish to express my thanks for the many courtesies extended to me while at work in the Muséum d'Histoire Naturelle, Paris, and especially to record my appreciation of the kindness of Professor E. Trouessart, of the Department of Mammals and Birds, who has so generously put the resources of his Laboratory at my disposal.

back of the head, is of medium length and tapers off towards the shoulders. The tail is short and drooping, the voice shrill and harsh. Neither male nor female bears spurs nor is the metatarsus feathered. The color of the plumage in general is a blackish or gray ground, marked with numerous conspicuous white dots. The feathers frequently appear to have more or less of a bluish or purplish wash. Certain feathers of the wings may show transverse white bars instead of the dots.

The black langshan cock (Fig. 2, Plate I) is of large size, weighing about ten pounds. The head, of medium size, with bright red face, bears a rather large, upright red comb, and well developed, pendant earlobes, likewise red. Rounded red wattles of medium length are also present. The neck is of medium length and well feathered, the hackles flowing well over the shoulders. The beak is stout at the base and well curved, dark brown in color shading to a pinkish hue near the lower edge. The sickles and tail coverts are long. The metatarsus is feathered down the outer side and in all males of the species bears a well developed spur. Furthermore, the outer toe on each foot is feathered to its extremity. In color, the neck, back, saddle, sickles and coverts are a glossy metallic black with greenish sheen; the breast, primaries, secondaries, tail, fluff; shank and toe feathers are of a duller black.

The hybrids (Figs. 3, 4, Plate I) in weight and size, approximate more closely to the male parent, averaging eight pounds in weight when three years old. In general configuration of the body they are about intermediate between the chicken and the guinea. The plumage and ornamentation of the hybrids, however, is more generalized than that of either parent. The head shows no trace of the elaborate ornaments of either the mother or the father but is plainly feathered clear to the beak. A slight trace of the guinea's bluish white face is still discernible in the immediate region of the eyes where a small area is bare except for a sprinkling of fine hair-like feathers. The head is long and rather cylindrical and merges into the long neck in such a way as to give it a decidedly snake-like suggestiveness: The beak, in color and shape, resembles more that of the guinea but is of somewhat greater curvature. The feathers of the neck and head are narrower on the whole and

of finer texture than the corresponding feathers of the cock. A longitudinal median fold bearing mostly minute white feathers hangs from below the throat as in the guinea. Although all five of the hybrids are male none bears spurs. The legs are strong and the metatarsus of each individual bears a few small feathers along the outer, side but much more sparsely distributed than the corresponding feathering of the male parent.

The breeder has assured me that when young they resembled young guineas but, with advancing age, came more and more toward an intermediate condition. I secured them shortly before they were three years old. Three of them were killed at the age of three years, one at the age of six, and the other is still (1909) alive in the Cincinnati Zoölogical Garden. Curiously enough the two which were kept alive at the Zoölogical Garden continued to approach more closely the chicken (male parent) type and at the age of five years and since that time, each has possessed a well developed pair of sickle feathers (Plate I, Fig. 4) similar to those which are so characteristic of the ordinary domestic cock. Some clew to the cause for the late appearance of such characteristically individual qualities of the male parent may lie in the fact that in hybrids, because of the initial incompatabilities which must necessarily exist between the two strange germplasms, the male plasm requires time to become adjusted to its new and strange environment. This is all the more comprehensible if, as I have maintained elsewhere,2 it is the egg chiefly which determines the fundamental animal form (i.e., such characteristics as are common to both the male and female of a given kind of animal), although both sperm and egg contribute the individual and specific peculiarities of the respective parents. I may add that a conclusion similar to this has since been arrived at by Conklin<sup>3</sup> as a result of his embryological investigations.

The voice of the hybrid is much louder and even more discordant than that of the guinea although it bears more resemblance to the latter. The hybrids were always extremely wild and never

<sup>&</sup>lt;sup>2</sup> Guyer, M. F.: Do Offspring Inherit Equally from Each Parent? Science, vol. xxv, no. 652, June 28, 1907.

<sup>\*</sup>Conklin, E. G.: The Mechanism of Heredity. Science, January 17, 1908.

became tame although frequently handled. When held they would struggle and try to bite and would discharge from the cloaca, time after time, great quantities of an offensive viscid liquid, apparently as a means of defense. In this connection it should be stated that dissection showed the cæcal appendages to be much larger than those of either parent form.

In general texture the feathers are more or less intermediate in structure but, although larger on the whole, perhaps resemble more those of the guinea. The tail, while less erect than that of the langshan, is vaulted and never droops like that of the guinea. The large quill-feathers of tail and wing frequently possess vanes which are black on one side of the rachis and more like the general hybrid plumage on the other. In general there is a tendency for the unexposed parts of the larger feathers to be black and for the exposed parts to be reddish or yellowish brown, more or less mottled with black. Traces of the white markings to be mentioned later are also frequently in evidence. In all of the fowls the first primary is white; in some the second and third are also white. This is a condition which is frequently found in the corresponding primaries of the guinea. The conspicuous white dotting so characteristic of the plumage of the latter is entirely lost. The feathers of the head and neck are mainly black although some of the hackles show a pronounced reddish or chestnut tinge. In two of the forms there is also a decided sprinkling of white feathers in this region. Furthermore these two show occasional white feathers at various places on the body and likewise a considerable number of reddish brown feathers which may be more or less mottled with black (Fig. 4, Plate I).

The striking feature in the plumage of all these hybrids is that most of the feathers exhibit a pronounced vermiculation of successive, narrow, whitish, U-shaped bands' which gives the plumage as a whole the appearance of being barred (Fig. 3, Plate I). It is to this feature that I wish to call especial attention. In three

<sup>&</sup>lt;sup>4</sup> These white bands are, of course, areas free from pigment and it is really the arrangement of the pigment between them that gives the characteristic appearance. Nevertheless, since there must be some positive inherent factor which causes them to remain unpigmented, they have been treated as positive characters throughout the course of the present paper.

of the fowls the general ground color is blackish and the vermiculations white, in the other two there is much of a reddish-brown or chestnut tinge to many of the feathers, involving also to some extent the whitish bands so that there is less contrast in the color markings (Fig. 4, Plate I; Fig. 9, Plate III).

These five guinea-chicken hybrids, however, are not the exclusive possessors of this curious pattern, for at the Museum d'Histoire Naturelle in Paris, there is a guinea-chicken hybrid, which bears a very similar white vermiculation on many feathers of the back, ventral surface, and sides (Fig. 13, Plate III) although the fowl itself has more feathers which are entirely white. This hybrid was donated to the Paris museum in 1854 by the London Zoölogical Society. It differs from all similar hybrids that I have seen, in the possession of a conspicuous beard of white feathers extending from ear to ear across the front of the throat. feathers on the top and back of the head, and on the back and sides of the neck are for the most part black and beyond being more plentiful they do not differ essentially from those of the other hybrids. The throat, in front, bears some white and some reddish brown feathers. Since there is no record of the breed of chicken used in making the cross it seems useless to speculate on the origin of the remarkable beard beyond suggesting that it is possibly derived from an individual of one of the breeds of chickens which are similarly bearded. A fuller description of this hybrid including a photograph has been given elsewhere.<sup>5</sup>

After viewing these various hybrids, the first question to arise is concerning the origin of this pattern of white U-shaped vermiculations common to them all. Manifestly it cannot be derived directly from that of either of the immediate parents, in the five individuals of which the parentage is known, since the cock is wholly black and the guinea spotted, although the latter may show some barring on certain of the feathers.

Turning to ancestral species we find that the common chicken is in all probability a domesticated form of the red jungle fowl of India, Gallus ferrugineus, to which our common black-breasted

<sup>&</sup>lt;sup>6</sup> Guyer, M. F.: La Livrée du Plumage chez les Hybrides de Pintade et de Poule. Bul Muséum d'histoire naturelle, Paris, February, 1909.

red game shows much resemblance. The reasons most frequently advanced for believing this are the fertility of the red jungle fowl and common fowl when crossed and the well established fact that the descendants of domestic fowls which have been running wild for a number of generations in certain islands of the Malay Archipelago have reverted approximately to this wild type. Furthermore, similar reversions may be effected by sufficiently mongrelizing different breeds of chickens.

The cock of Gallus ferrugineus has a naked red face and throat, wattles, a well-developed, serrated, red comb, and whitish earlappets. The feathers of the crown, upper back, upper wings and rump are orange red, the remainder of the back mainly purplish red. The tail, wings and under parts are glossy greenish-black. The outer margins of the primaries are yellowish, and of the secondaries brownish. The hackles and long tail plumes are replaced by shorter black feathers during the summer.

On the hen, wattles, spurs and elongated retrices are lacking, and the comb is but feebly developed. The feathers of the crown are reddish, and of the upper back and wings, yellowish striped with black. The remaining plumage is mostly of some shade of reddish or yellowish brown mottled with black. The reddish hue of the brown is particularly in evidence on the breast and foreneck. The shafts of the feathers are of light color and on the breast especially give much the effect of light-colored stripes.

From this description it will be seen that reddish-brown or reddish-brown mottled with black is a color much in evidence in the plumage of Gallus ferrugineus. It will also be recalled that there was a considerable outcropping of the same color in the guinea-chicken hybrids. There is, therefore, apparently a return in certain feathers of the hybrids to the primitive ancestral color of Gallus. The return to this same color is of frequent occurrence in the plumage of other hybrids where the domestic chicken is involved. Thus, in a peafowl-chicken hybrid (Museum d'histoire naturelle, Paris) it is the prevailing color although there are also a number of white or partially white feathers present. The recurrence of this color may also be seen in several pheasant-chicken-hybrids which I have examined. It is of very frequent occurrence,

moreover, in mongrels of our barnyard fowls. It is a well recognized fact, indeed, among breeders that this fundamental reddish cast of feather is a very difficult one to breed out of fowls entirely.

It is possible, furthermore, that the black neck-feathering of a number of pronounced hybrids which I have examined, such as guinea-chicken, peacock-chicken, and pheasant-chicken, all of which are males, is a return to a condition similar to that found on the neck of the male of Gallus ferrugineus from June to September.

But granting that in the respects just enumerated there is a resemblance to the wild type of Gallus ferrugineus, still this sheds no light upon what we have recognized as the most striking characteristic of the plumage of the guinea-chicken hybrids; namely, the distinctive white vermiculations of the feathers.

Before taking up a discussion of this feature it will be necessary to recall a few points in the systematic relationships of certain members of the family Phasianidæ. There are, according to Evans,<sup>8</sup> really three distinct sub-families of this group: (1) The Numidinæ or guinea-fowls; (2) the Meleagrinæ or turkeys; and (3) the Phasianinæ or pheasants (including domestic fowls and peafowls), partridges and grouse although for convenience the partridges and grouse are ordinarily set apart as distinct groups. It will be seen from this classification that, inasmuch as the guinea parent of the hybrids under discussion belong to one sub-family and the other parent to another, a guinea-chicken hybrid is a more pronounced cross than a hybrid between peafowl and chicken, or pheasant and chicken, to say nothing of hybrids between the various kinds of pheasants in the more restricted sense.

In the sub-family Phasianinæ is found an interesting group known as the peacock pheasants (Polyplectron) which is regarded by systematists as intermediate between the peafowls and the pheasants (including Gallus) in the narrower meaning of the

<sup>&</sup>lt;sup>6</sup> Ewart, J. C.: The Pennyicuk Experiments, 1899. Darwin, Ch.: Animals and Plants under Domestication.

Tegetmeier, W. B.: On the Principal Modern Breeds of the Domestic Fowl The Ibis. Sixth Series, 11. ii, 1890.

<sup>\*</sup> Evans, A. H. The Cambridge Natural History; Birds vol. ix, p. 198, 1899.

Grant, W. R. Ogilvie: Birds in the Dept. of Zool., British Museum, p. 22, 1905.

term. They are found in the Indo-Malayan countries and the islands of Sumatra, Borneo and Palawan. Upon close inspection of the group, one is indeed impressed with this intermediacy, and can readily see as regards plumage at least how such forms as the peafowl on the one hand and the pheasants (and among them Gallus) on the other, could have diverged from some such generalized form as is found in the simpler species of Polyplectron. One sees in them in its incipiency, as it were, characters which only attain to their fullest expression in these other groups.

Among the several species of Polyplectron itself, indeed, may be found interesting examples of progression in color pattern. In P. chalcurus, for example, one finds on the back and tail (Fig. 2, Plate II; Fig. 8, Plate III) a simple U-shaped barring of alternate brownish-black and reddish-brown bands. In certain other forms of Polyplectron one sees an increase in the light color of the lighter bands and a transition of each of these unbroken curved bands to a series of dots (Fig. 6, Plate III), of which, however, the transverse arrangement is still plainly apparent. In still other members of the group, the shifting of the pattern has gone so far on many feathers of the wings, saddle and tail that its transverse nature is almost wholly obscured, although on the breast and neck of some of these species, as P. thibetanum, or P. germaini it is distinctly discernible. In these two species, in fact, the males especially show a definite "cuckoo" marking very similar in appearance to that of certain breeds of domestic fowls such as the Plymouth Rock or the Dominique. It is inferred that the direction of the change has been from the transverse bar toward the uniform dotting, rather than the reverse, because such an order is parallel to the other specializations of the plumage, and because, furthermore, the males, which in most species of Polyplectron are phylogenetically in advance of the females, exhibit the dotting while the females are still in the barred condition. Then, too, in the development of the plumage of the individual the stage of barring precedes that of the dotting.

Again as regards ocelli or "eye-spots" in P. chalcurus which appears to be the most generalized species, one finds no ocellation. The only hint of what is to be realized by the more special

ized members of the group is found in a pronounced purplish and greenish "metallic" coloration present on certain feathers of the In the male of P. emphanes, while there are numerous green metallic iridescent areas on the feathers of the upper wings and back, they have not yet progressed to the condition of being definite ocelli, although on the tail of this same individual there are two transverse bands (the one on the retrices, the other on the upper tail coverts) of ocelli. Still a step in advance, in the male of P. thibetanum, Gm. (P. alboocellatum, cuv.; Type, Mus. d'hist. nat., Paris), the smaller feathers of the wings and the feathers of the interscapular region bear distinct small purple ocelli ringed successively with black, light brown and white. The tail is also banded with ocelli. In the male of P. germaini the wing-coverts and back bear numerous green ocelli. The female of this species, as usual less advanced phylogenetically than the male, has the ocelli of the body much less distinctly marked. Moreover, they are entirely missing from the tail of one specimen examined and more or less obscurely represented on the tail of the other.

Outside the group polyplectron all are familiar with the remarkable occillated "train" (the enormously developed upper tail coverts) of the peacock. Again in the argus pheasants is found a pronounced development of occili. Thus, this same tendency is seen to crop out in group after group of the Phasianinæ which, judging from the intermediate nature of Polyplectron, could have had some primitive member of this genus as the common ancestral form.

With this thought in mind it is interesting to examine still farther the species P. chalcurus (Type, Mus. d'hist. nat., Paris) of Sumatra. (It is called P. (chalcurus) inocellatus in the Cambridge Natural History, vol. ix, p. 208). This species could, indeed, stand near what might have been the common ancestor of the peafowls, jungle fowls, and pheasants. The generalized form of the bird is obvious (Fig. 2, Plate II). Even the male, of which Fig. 2 is a photograph, is devoid of all special head ornaments such as comb, wattles, ear lobes, crests, etc., which are present so commonly in the males and often in the females of many gen-

era of Phasianinæ. The head is simply and completely feathered except for a small region in front of and behind the eyes. In most of the other species of Polyplectron, in fact, there is simplicity of head feathering although in certain species the males have modest crests. Thus P. emphanes has a small crest, and a white face reminding one somewhat of the peacock although in P. emphanes the white is in the feather. P. schleiermacheri of Borneo has a crest which curls forward. While bearing in mind this simplicity of head covering in Polyplectron, it should be noted also that in all the hybrids from widely divergent ancestry which I have examined among the Phasianidæ such as peacock-chicken, guineachicken, and pheasant-chicken, there has been a marked return to some such simple type.

Returning to a discussion of the primitive features of P. chalcurum, we find, furthermore, that the color of its plumage might be regarded as a primary type from which the hues and patterns of the Phasianinæ in general could have been derived. Looked at from a distance, the general effect is reddish brown with curve barring on the back and tail (Fig. 2, Plate II). The breast is even more reddish in color, and plain. An examination of a single feather of the back (Fig. 8, Plate III) shows the pattern to be that of a series of alternate darker and lighter colored crescents or U's of which the dark ones are almost black and the others a light reddish brown—a black and white feather washed, as it were, with reddish brown. The lighter colored U's are from four to five in number on most of the feathers of the back, rump and abdomen.

It will be seen from an inspection of this pattern that the more transverse markings of its kinsmen (Fig. 6, Plate III) in the genus Polyplectron could readily have been derived from it by more or less of a suppression of the arms of the U. On the other hand, if the arms became lengthened out there would be formed an elongated pattern approaching a stripe. It is precisely such a condition as this that is present in the feathers of many pheasants (Fig. 1-5, Plate III).

In P. chalcurus itself, in fact, on the wing coverts one finds all gradations in the transition to this elongated, pheasant type of

marking although the color contrasts are too slight to be shown by the camera. However, since one finds on many of the wing coverts of Gallus ferrugineus a very close approximation to this scapular marking of P. chalcurus, a feather from Gallus has been chosen for purposes of photography (Fig. 7, Plate III). While the color contrast even in it is not pronounced, still one can readily make out a narrow, light colored border, then a narrow, black zone running the full length of the web on either side, and within this still further indications of alternating light and dark zones. The transverse nature of the bands is still faintly in evidence near the tip of the feather. The striping or lacing is really much more in evidence in the females of Gallus sonneratii and particularly of Gallus varius, but G. ferrugineus has been chosen for illustration because of its presumably closer relationship to domestic fowls.

The black mottling on various other feathers of Gallus ferrugineus is very similar also to that of certain feathers of Polyplectron chalcurus. Taking all in all, one can see how the color markings of the former could have been derived from a more primitive type such as apparently exists in the latter.

That Gallus is considerably in advance of Polyplectron chalcurus seems evident when one takes into account the increased ornamentation and specialization of the plumage of even the female of Gallus, to say nothing of the head ornaments (comb, wattles, etc.) and other special features of the male. Still other primitive characteristics of P. chalcurus are the prevailing reddish brown color on the breast; and the simple condition of the iridescence of the tail. Again, should be noted the tendency for the shaft of many of the feathers of the back and ventral surface of the body to be light colored although in certain regions they may be darker. They vary from a reddish yellow to a deep mahogany. The light color of the rachis is much more in evidence in the jungle fowl, especially the female, and is also a frequent occurrence in most pheasants. In fact considering the evidence as a whole one is inclined to believe that as regards color pattern at least Polyplectron chalcurus stands very near the ancestral form whence sprang the Phasianinæ.

There is only one point in which Polyplectron seems to be highly specialized and this is the possession by the males of more than one pair of spurs. These spurs may vary in number on the two legs of the same individual. An increase in the number of spurs, however, is by no means a peculiarity of this genus. The male of Chrysolophus pictus, the golden pheasant, generally has two spurs on each metatarsus; the male painted-spur fowl (Galloperdrix lunulata) may have two or three pairs of spurs; and in the blood pheasants (Ithagenes) the male may have as many as four pairs. The tendency, therefore, is not confined to any one species or genus of the Phasianinæ. It could be argued, indeed, that this very irregularity in the spurring of Polyplectron is a more primitive condition than the regular and stable condition of such a genus as Gallus.

In any event, we find in the Phasianinæ as a whole a tendency in color pattern toward the development of more or less of a crescentric or U-shaped barring which in certain members of the group approaches to a striping.

As regards the hybrids under consideration, however, there still remains the question of the guinea ancestor.

The guineas (Numidinæ) found only in Africa and the Madagascar region seem to stand as more or less intermediate between the Phasianinæ of the Indian and Palæarctic regions and the Meleagrinæ of America. An examination of the color pattern of normal specimens of the domestic guinea or of its wild progenitor Numida meleagris, of which the coloration is essentially the same, gives us little direct clew to the marking of the hybrid plumage. Outside the genus Numida, however, one finds a very significant color pattern in the rare West African form, Agelastes meleagrides (Fig. 1, Plate II), which ranges from Liberia to Gaboon. The head and neck of this fowl are bare and of bright red color. The neck becomes whitish towards its base and terminates in a broad zone of white feathers. The plumage in general is blackish, vermiculated with fine white transverse markings. The narrow outer webs of the remiges have whitish margins.

While the white vermiculations can be seen upon closely examining any of the exposed black feathers, they are very fine and in-

conspicuous on the back and are most distinctly visible on the feathers of the sides and ventral surface. Fig. 15, Plate IV, is a photograph of a feather from the ventral surface of the fowl. By comparing it with Figs. 9, 11, 12, 13, Plate III, it will be seen that it resembles somewhat in the wavy nature of its bands the marking of the hybrid feather.

The question confronts us as to whether any connection can be traced between this color pattern of Agelastes meleagrides and that of Numida meleagris, which is the real ancestral species of the hybrids.

In the most conspicuously marked feathers of N. meleagris one sees only the characteristic white dots on a dark background (Fig. 28, Plate IV), but when certain feathers of the neck, back and breast are examined carefully (Figs. 16–27, Plate IV) considerable trace of barring, especially towards the base of the feather, may be found. These feathers have not yet progressed to the stage of complete dotting. However, in a typically spotted feather (Fig. 28, Plate IV) one may usually see in the arrangement of the dots a trace of the transverse pattern. Even on the exposed parts of some of the wing feathers of certain individuals (Fig. 1, Plate I) there are transverse white bars. Furthermore, on certain of the upper neck feathers there is little trace of the ordinary larger white dotting, but in its stead there exists a series of finer white curved markings (Figs. 20, 21, Plate IV) which approach more nearly in appearance those of Agelastes (Fig. 15).

I have found still further interesting examples of this barring in two unusual specimens of Numida meleagris at the Mus. d'hist. nat., Paris. These two fowls resemble closely a form that I once obtained in a Cincinnati market. The characteristic dotted color pattern has failed to develop except on a few flight feathers and on certain feathers of the ventral surface of the body. Many of the exposed feathers have, however, a salt and pepper effect in coloring, due to the presence of minute specks of white on the darker background (Figs. 22, 25, 26, Plate IV). This white dotting can, upon close inspection, be resolved into more or less of a definite series of vermiculations approximating those of Agelastes meleagrides. In still other feathers of each of the three fowls, a

distinct and broader V-shaped white barring is well developed near the base of the feather (Fig. 25, Plate IV) as in certain feathers of the normal domestic guinea already described.

Indications of the finer Agelastes-like barring are to be found on many feathers of the neck in other species of Numida and, what is of further significance, they are well developed on certain feathers of the upper back and lower neck of a species belonging to a still different genus, namely Acryllium vulturina. This species is profusely decorated with white dots of somewhat smaller size than those of Numida. However, on some feathers of Acryllium, certain feathers of the wings, for example, there are pronounced longitudinal stripes. This is evidently a progression beyond the condition of spotting because, as is evidenced in the series of young in the Muséum d'histoire naturelle, the striping appears later instead of earlier in the development of the color pattern of the adult plumage. In the half-grown young, for example, the feathers display a wholly barred pattern except for a few dots on tail and wing which have evidently been derived from earlier bars. white bar in Acryllium vulturina of which the dots are a product is intermediate in breadth between the slight marking of Agelastes meleagrides and the heavier bar of Numida meleagris.

An examination of such a feather as that of Numida meleagris pictured in Fig. 28, Plate IV, shows how the dotting derived from these bars passes readily over into a pattern of longitudinally arranged dots which in later evolution might become stripes, a progression which has already become an accomplished fact in such feathers of Acryllium as are striped.

Taking this evidence all together one seems justified in concluding that in certain of the guineas (Agelastes, Numida, Acryllium) there is or has been a primitive white barring of the feathers and that in the three peculiar individuals recently under discussion, more or less of a reversion to this pattern has taken place, or in other words, the color pattern has for some reason stopped short of its fullest development. It seems likely, furthermore, that in these regressive individuals, we find traces of two types of barring, namely the finer barring (Figs. 22, 25, 26, Plate IV) similar in appearance to that of Agelastes meleagrides, and a heavier barring,

probably derived from the former, such as is found at the base of the feathers of the regressive forms and on certain of the wing feathers and at the base of many other feathers of the ordinary domestic guinea.

There is evidence, furthermore, in the plumage of Numida meleagris that in the formation of the heavier barred type only every other one of the bars of the primitive pattern have been thickened (cf. Figs. 16, 17, 19, 20, 25, Plate IV), the alternate bar tending to disappear.

The sequence of the pattern in the guineas would seem to be from what originally was probably a simple irregular specking with white, to the narrow fine vermiculations such as now exist most characteristically in Agelastes meleagrides followed by a broader barring of white as evidenced in certain feathers of the domestic guinea. This barring has been largely transformed or is in process of transformation into a conspicuous white dotting as seen in Numida meleagris and Acryllium vulturina. Certain feathers of Acryllium are even a step further in advance since the dots have seemingly merged to form a longitudinal stripe.

Thus, it is an interesting fact to note that in both the sub-families Phasianinæ and Numidinæ, in various comprehensive groups of genera and species, there are certain basic tendencies for particular elements of the coloration, such as the formation of eye-spots, barring, and the like, to follow along definite paths of development. When arranged with reference to one of these elements, such for example, as barring, which is one of the most universal, instead of possessing distinct and unrelated markings, the different species in a given group are seen to be standing merely at different levels in the development of one, or at most a few, continuous progressions in the development of the special pattern in Since when so grouped the gradation in pattern is as question. much in evidence between collateral kinsmen as between those of direct lineage, one can only conclude that the bias toward a particular line of patterns is the product of fundamental protoplasmic peculiarities implanted in the group as a whole. In a few instances the characteristic design may be wholly obscured; in many cases it has been warped and twisted, as it were, possibly by natural selection, it may be by sexual selection, perhaps, directly or indirectly, by other factors of the environment; nevertheless, the pattern tends to reveal itself in some stage of the progression, or progressions, which are characteristic of the group as a whole.<sup>10</sup>

The writer does not care to enter into a general discussion of the vexed question as to which is phylogenetically the older pattern, the stripe or the bar. It seems plausible to suppose, as some do, that all groups of birds have not followed the same order in the development of their respective patterns and that what is archaic in one group is not necessarily so in some other. While the first plumage in the young of Gallinæ is striped, as pointed out by Darwin, still we must bear in mind that this striped appearance is due to the relative arrangement of deeply pigmented and of colorless feathers respectively which are themselves unstriped. The underlying physiological conditions which produce such a pattern, therefore, must be very different from those which produce an individually striped or barred feather, since one has as its basis an arrangement of the feathers on the bird, the other, a series of processes in a particular feather.

On such feathers as are wholly or partially concealed one would naturally look for the phylogenetically older color pattern, if one admits of the theory of selection in any degree, since these hidden parts would not be subject to the disturbing influences of natural or of sexual selection, which so far as color patterns are concerned, require the parts affected to be visible. It is precisely in such places that one still finds most of the traces of barring in the domestic guinea (Figs. 17, 23, 27, Plate IV) and in the pheasant (Fig. 14, Plate III). Furthermore, in such hidden regions is found the principal portion of the color pattern of the domestic guinea which persists and even shows evidences of augmentation in regressive

<sup>10</sup> Inasmuch as the question of evolution in definite directions irrespective of utility does not fall primarily within the scope of this paper, the reader is referred for discussions of the subject to the original papers of such investigators as Hyatt, Escherich, Haacke, Cope, Eimer and Whitman. See especially: Eimer, Th.: On Orthogenesis, Open Court Pub. Co., 1898. Whitman, C. O.: In the Proceedings of the Congress of Arts and Science, Universal Exposition, St. Louis, 1904, vol. v. Also, Bul. Wisconsin Acad. of Sciences, Jan., 1907.

individuals of the latter species (Fig. 25, Plate IV; note the markings along the shaft).

And now comes the question as to what conclusions may be drawn with reference to the characteristic color pattern of the guinea-chicken hybrids under discussion. Is it reducible to the pattern of such a form as Polyplectron chalcurus or to that of other pheasants or does it rather return to one more like that of Agelastes meleagrides of the guineas? The truth would seem to be that it reverts to none accurately, but shows the mingled influence of both lines of ancestry. The united pull of a primitively barred ancestry on each side has been sufficient to make this tendency to barring stronger in these particular individuals than all others in the formation of the pattern, but the pattern is, as it were, a composite.

An examination of such figures as Figs. 9, 11, 12, 13, Plate III, shows that the vermiculations are less numerous and proportionately broader than those of Agelastes meleagrides (Fig. 15, Plate IV), even when one takes into consideration the fact that Agelastes is a smaller bird, and has in consequence smaller feathers bearing a more delicate pattern. Furthermore, the bands are more pronouncedly U-shaped, the arms of the U being much lengthened and tending to run parallel to the shaft in most of the hybrid feathers. Again, in such feathers of the domestic guinea as show traces of the broader barring, while the white bands are of approximately the same breadth as those of the hybrid, they are less wavy and they are proportionately closer together (Figs. 23, 25, 27, Plate IV); moreover, they never run approximately parallel to the shaft of the feather. This last trait, indeed, is more like that which is characteristic of the pheasants as may be seen by examining Figs. 4, 5, 10, Plate III, of feathers from ordinary pheasants bought in the Paris market or Figs. 1-3, Plate III, of feathers from pheasant hybrids. On the other hand the number of stripes in the pheasant feathers is fewer in the great majority of cases than in those from the guinea-chicken hybrids and they are, on the whole, of more even outline.

In the wavy character (vermiculation) of the white pattern of the hybrids, the guinea type as exemplified in Agelastes meleagrides is most closely approximated. However, taking for consideration feathers of the lower back region of the latter and of the hybrids respectively, one finds that the ten to twelve vermiculations of Agelastes have in the hybrids apparently yielded to the restraining influence of the lesser number of bands found among pheasants in general, with the result that in the corresponding feathers of the hybrids three to six U-shaped bands are present.

As already noted the white band of the hybrid feather, with few exceptions, crosses the shaft at less of an acute angle than in most pheasants. This is a feature which might be reduced to the type of either such a form as Polyplectron (Fig. 8, Plate III) or Agelastes (Fig. 15, Plate IV). From the fact that most of the pheasants tend to have the sharper-angled or V-shaped marking, it would seem that the departure from this type is more likely to be due to the stress of the less remote guinea ancestor of Agelastes-like color pattern than to that of some ancestor as distant from Gallus as a primitive Polyplectron would be, although it is not impossible that both have been influential.

The case is by no means unequivocal, because on a few obscure feathers of the common pheasant may be found markings (Fig. 14, Plate III) that make almost as much of an angle with the shaft as do those of the hybrids and a few pheasants show commonly a broad U-shaped marking in all plumage. These white markings (Fig. 14, Plate III) of the common pheasant will be seen to resemble also rather closely those of the hybrids in other respects. They tend, however, to be broader, to have an outline that is slightly more jagged, and to meet the shaft at more of a sharp angle. The feathers which show them are from the scapular region and when in place are hidden almost entirely by overlying feathers which bear a brighter and presumably a more recent color pattern.

For the foregoing work of comparison, feathers other than the larger ones of wing and tail have been used because they are more constant in their characters. As to certain of the tail and wing feathers of the hybrids, they show also white vermiculations which vary in number with the length of the feather. There may be as many as twenty on some feathers and they tend to be more transverse than those of the body feathers. The pattern is very similar

to that found on the tail feathers of various pheasants and particularly of certain hybrid pheasants which I have examined. The pheasants in general have much more strongly developed tails than the guineas and their characters have dominated in the tails of the hybrids. As to the white primaries of the hybrids, it has already been stated that such are also of frequent occurrence in the domestic guinea, and it was noted also that in Agelastes meleagrides the outer margins of the primaries are whitish.

To sum the matter up briefly, in the guinea-chicken hybrids under discussion, it would appear that the characteristic color pattern of white U-shaped vermiculations on a dark background is a return to a generalized type of color marking that is more or less recognizable in various groups of the sub-families Phasianinæ and Numidinæ. The immediate pattern as seen in these hybrids is seemingly a composite of primitive loop-like bands as exemplified in Polyplectron chalcurus together with the prevalent stripe of the more specialized pheasants, on the one hand, and, on the other, of the numerous transverse vermiculations of an ancestral color pattern approximating that of Agelastes meleagrides. The short black feathers of the neck found in all of these pronounced hybrids seem as already indicated, to approach more nearly the condition of the feathers found on the neck of Gallus ferrugineus during the summer months. The even feathering of the head and loss of ornamentation appears, however, to be yet more primitive, approaching the simpler types of Polyplectron. The total lack of spurs in the genus Numida and their presence only in the males of Gallus has sufficed to bring about their suppression in the hybrids even though the latter are male. The frequent recurrence of reddish-brown or brown mottled with black in the plumage of such hybrids as guinea × chicken, pheasant × chicken, and peacock × chicken is presumably a return to a condition such as exists in Gallus ferrugineus or in still more primitive types such as Polypectron chalcurus. If one could express the whole matter in one phrase it would be that the return in each regressive feature seems to be more to primitive fundamental states or conditions common to a number of allied groups than to any particular recognizable ancestor. To the writer, at

least, this would seem to be the most reasonable interpretation that presents itself. It might be urged, of course, that as regards the white banding, for instance, it is simply a case of "new variations which happen to hit the old mark." But why in the very limited number 11 of known guinea-chicken hybrids has it "happened" in five instances, in America recently, and earlier in a similar hybrid in England which dates from 1845? And why as regards various other features of pronounced hybrids have so many "old marks" been hit? Why, indeed, as pointed out by Darwin<sup>12</sup> on the authority of Tegetmeier, is there a very general tendency among gallinaceous birds for a barred pattern to appear when pure-bred forms are crossed? If we argue that the very nature of the protoplasm of the two species hybridized limits the number of possible variations and hence makes it probable that a given variation may be hit upon repeatedly, in admitting this markedly restrictive and hence determining character of the protoplasm do we not admit that the phenomenon is hereditary (that is, reversionary) in nature rather than fortuitous?

The great question, of course, which presses for solution is how such returns come to pass, and it must be admitted that concerning the real nature of the processes we are almost as much in the

<sup>11</sup> Still farther confirmatory evidence in this particular has come to my knowledge since my manuscript was sent to press. I have had the opportunity of examining seven other guinea-chicken hybrids; two in the South Kensington Museum of London, and five in the American Museum of Natural History in New York City. Of the former, one had been received from Brazil in 1899 and the other from Kalka, India, in 1902. The superficial feathers of the Brazilian form were white, splashed somewhat with red, but when these were pushed to one side various underlying feathers showing pronouncedly the characteristic chevronlike markings were disclosed. The Indian form, of which the plumage was heavily washed with reddish brown, likewise exhibited the chevron barring, especially on the underlying feather.

The five specimens in the American Museum of Natural History were the donation of Dr. Juan Vilaro of Cuba, according to whom such hybrids are by no means rare in Cuba. (Hybrids between the Game Cock and the Guinea Fowl, by Juan Vilaro, M.D., Bul. Am. Mus. of Nat. Hist., vol. ix, article xviii, August 21, 1897.) Four of these birds, although all of separate parentage, show at first glance an abundance of the markings in question. The fifth bird is white, the offspring of a pure white guinea-fowl and a white hen. Notwithstanding the lack of pigment, many of the feathers of this white hybrid, upon close scrutiny, show the presence of the chevron-like bands across the vane, thus demonstrating that the characteristic barring is due primarily to structural features of the vane itself, the pigment being a secondary factor which by its presence serves to emphasize to the eye certain regions of the fundamental structural pattern.

<sup>13</sup> Darwin, Ch.: The Variation of Animals and Plants under Domestication. Vol. i, p. 295; vol. ii, p. 27.

dark today as in the time of Darwin, despite the new impetus given to the study of the problems of hereditary through the renaissance of the Mendelian principles. In such cases, as I have suggested elsewhere, we appear to get entirely outside the pale of minor so-called "unit characters" which may be juggled to and fro from generation to generation, and to face an entirely different proposition; namely, that of the more fundamental and the more superficial respectively, of the more permanent as against the more fluctuating. The upsetting of a breed by hybridizing or mongrelizing may bring about in successive crosses, seemingly, a more or less systematic reversal of the process of construction of a given pattern; the regression proceeding for the most part from the latest additions to the less recent until finally a form closely approaching that of the primitive ancestor appears. Thus, for example, may be cited the results of Ewart<sup>14</sup> who found that in pigeons, when owl and archangel were crossed, the latest colors added by the fancier were not reproduced but older and simpler colors became visible again. When this mongrel was further crossed with a white fantail all recently acquired colors were lost and the primitive ancestral type as seen in the wild rock pigeon was almost completely reproduced. Crosses from such widely separated forms as guineas and chickens seem to undo at one stroke most of the recent accumulations of each species.

Possibly part of the explanation lies in this, that having diverged from a common stock the parents of such hybrids have certain fundamental features and tendencies in common, which as a result of the physiological processes of growth and development can come ultimately to a definite objective expression as they did in the original common ancestor. On the other hand, certain traits acquired since their divergence from the parental stock have become so dissimilar and incompatible in the two forms that as a result of hybridization they annul one another and thus permit this unmasking, as it were, of old characters. Doubtless certain characters not represented in the opposite species or certain non-antagonistic characters may also manifest themselves.

<sup>&</sup>lt;sup>18</sup> Guyer, M. F.: The Insufficiency of the Chromosome-Theory of Heredity. Proceedings of the International Congress of Zoölogista for 1907.

<sup>16</sup> Bwart, loc. cst.

The supposition that old characters reassert themselves does not necessarily imply that they have been lying dormant in an isolated particle in the germ-plasm for a series of generations. It is just as possible that, as regards their objective expression, they have simply been obscured as the result of more recently developed modifications in their own substance. Or again, it is equally plausible to suppose that the earlier expression was the result of the union or special grouping of certain complimentary elements which have become separated or deflected by the newer acquisitions of the species and that with the suppression of these later additions the older groupings have been restored.

It is evident how this could be true particularly with such features as color where a very slight physical or chemical change might be optically expressed as a very pronounced difference in the color pattern. Thus the arrested development of a green-making superstructure may yield a yellow instead of green color. Indeed with apparently slight alterations one may get as great contrasts as red and green, as illustrated in certain parrots where, while the adult males are intensely green, the young males are red, resembling more the females, which are bright red.

Again, gloss and iridescence of feathers, it is known, are a matter of the structure of the horny coating of the feather rather than one of pigment. Since this physical structure is not exactly the same in the different species of birds that exhibit the phenomena, even where both ancestors of a hybrid have much iridescence the hybrid itself may be of plain dull colors, an example of which I have before me, in a peacock-chicken hybrid. One can only conclude, therefore, that the two different types of structure have annulled one another. Of all the hybrids of markedly divergent parentage which I have examined, in fact, not one shows more than a faint trace of iridescence although most of them as peacock × chicken, and pheasant × chicken, have progenitors on each side which display it to a considerable degree.

In the guinea-chicken hybrids, as we have seen, all the parental ornamentations of head and plumage have been suppressed. In color, only certain fundamental pigments and structures in a measure common to both groups have appeared, for the most

part, such as an abundance of black pigment, more or less of a reddish coloring matter, and the white pattern already described which in general alternates with the pigmented areas. White in feathers, as is well known, is not due to a pigment, but is the result of the diffractions and reflections of light from innumerable minute interstices. Since the pattern in question is prevalent in some of its modifications in both guineas and pheasants it has not been swamped when two such otherwise divergent forms as guinea and chicken have been merged, but the underlying structure necessary for its appearance has persisted in these particular cases and the pattern has come once more into evidence through the suppression of the more recent features which obscure it in the normal parent forms. The very fact that this modified striping or barring is met with even yet in so many Phasianinæ and Numidinæ shows that it is a fundamental structure which has not been seriously disturbed by later acquisitions.

### PLATE I

- 1 Domestic guinea-hen (Numida meleagris).
- 2 Black langshan cock.
- 3 Guinea-chicken hybrid, three years old.
- 4 Guinea-chicken hybrid, six years old.

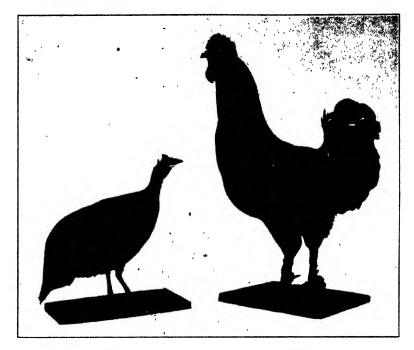


Fig. 1 Fig 2

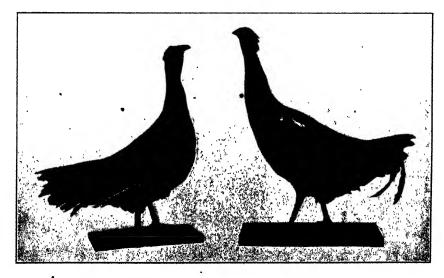


Fig. 3
The Journal of Experimental Zoology, vol. vii.

Fig. 4

### PLATE II

- I Agelastes meleagrides, showing the white vermiculations of the feathers. Compare this figure with Fig. 3, Plate I.
  - 2 Polyplectron chalcurus, showing the U-shaped barring of the feathers on the back.

To show properly the color pattern, it has been necessary to make these two photographs considerably larger in proportion than those of Plate I. Agelastes is really only about three-fourths the size of the domestic guinea and Polyplectron is still smaller.

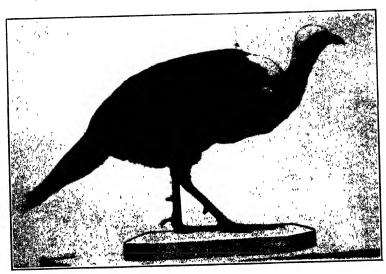


Fig. 1

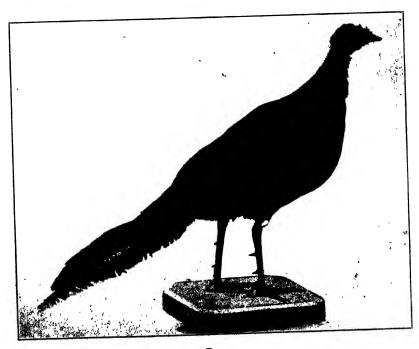


Fig. 3
The Journal of Experimental Zoology, vol. vii.

#### PLATE III

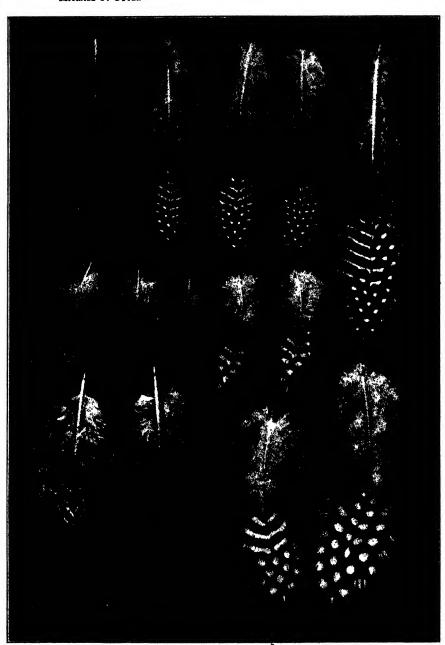
- I Feather from the wing of a hybrid pheasant (Gennæus nycthemerus × G. lineatus).
- 2 From the wing of a hybrid chicken × pheasant.
- 3 From the back of another hybrid chicken X pheasant.
- 4, 5. From the back of Phasianus colchicus.
- 6 From the breast of Polyplectron germaini.
- 7 Scapular of Gallus ferrugineus.
- 8 From the rump of Polyplectron chalcurus.
- 9 From the rump of guinea-chicken hybrid I.
- 10 From the back of Phasianus colchicus.
- 11 From the rump of guinea-chicken hybrid III.
- 12 From the back of guinea-chicken hybrid, IV.
- 13 From the rump of guinea-chicken hybrid, Paris museum.
- 14 Hidden scapular of Phasianus colchicus.



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#### PLATE IV

- 15 From the breast of Agelastes meleagrides.
- 16, 17, 18 From the back of the domestic guinea (Numida meleagris).
- 19 From the rump of the domestic guinea.
- 20, 21 From the neck of the domestic guinea.
- 22 From the neck of a regressive domestic guinea.
- 23, 24 From the breast of the domestic guinea.
- 25, 26 From the back of a regressive domestic guinea.
- 27, 28 From the back of the domestic guinea.



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### HEREDITY OF THE RACE-CHARACTERS UNIVOLT-INISM AND BIVOLTINISM IN THE SILKWORM (BOMBYX MORI)

#### A CASE OF NON-MENDELIAN INHERITANCE

#### ISABEL McCRACKEN

In the spring of 1905, during the progress of certain breeding experiments with the silkworm (Bombyx mori) to determine the hereditary value of certain larval characters, an unlooked-for phenomenon occurred. This was the hatching, some ten or fifteen days after the eggs were oviposited, of a number of broods of eggs that were supposed to be destined to lie over the winter and hatch in the following spring.

During the month of April (1905) some 210 inter- and cross-matings were made between moths that had been reared through the spring from "recorded" 1904 parents. After the matings were made, the mating boxes, small manilla paper boxes in which eggs were oviposited, were set aside to await removal to winter quarters. Ten days later the boxes were examined in order to remove the dead moths and to note the condition of the eggs. It was observed that the eggs represented two conditions. In many boxes, these had the gray appearance of the normal univoltin egg. In six boxes, however, the eggs, though alive, were of a dull yellowish appearance.

The univoltin race of silkworms produces but one generation during the year, a spring generation, from eggs that have wintered. When first oviposited, the univoltin egg is yellow, very light yellow if the larva or silkworm is destined to spin a white cocoon, a much deeper yellow or golden if the silkworm is destined to spin a yellow cocoon. About 24 hours thereafter, the

egg changes to a muddy or dull yellow tone which soon gives way to a strawberry pink and later to a characteristic gray.

The bivoltin race of silkworms produces two generations during the season. The eggs of the spring moths fail to go into the gray resting condition of the univoltin egg but issue after ten or fifteen days, during which time they remain of the dull yellow or slightly pinkish appearance while the young embryo of the summer generation is developing.

The occurrence of the bivoltin in the univoltin race is occasional but rare. In the thousands of broods that have been reared in our breeding rooms since 1903 it has occurred a number of times. Its occurence, I look upon as a reversion to an ancestral condition, mainly from the fact that the most nearly allied wild silkworms of China and India recorded by Rondot in "Les Soies" are bivoltin. Hutton¹ also gives some evidence for this view. As such, I am following up its behavior in heredity, for comparison with results herein recorded. It is hoped that further experimentation with this material will show what influence, if any, this known sporting or reversionary tendency of the univoltin race has upon the trend of heredity in experiments involving uni- and bivoltins.

The experiments recorded in the present series were carried on with offspring of pure univoltin and pure bivoltin stock.

The six masses of eggs of the 1905 lot of second generation hybrids that were noticed to remain in the dull yellowish condition as previously noted were subjected to a day-to-day inspection until, on the 13th and 14th days they were observed to hatch and a second generation or "summer" brood of larvæ appeared. Thus the significance of their not having entered the gray condition.

The 1904 records of these six broods were then looked up. These records showed that in each of the six cases, one (the female) or both parents had come from the identical spring brood. The 1904 female parent of this spring brood was a univoltin salmon-colored cocooner from a banded or "zebra" larva reared

<sup>&</sup>lt;sup>1</sup>Hutton Thomas: On the Reversion and Restoration of the Silkworm. Transactions of the Ento. Soc. of London, p. n. s. vol. iv, 1864, pp. 143-173.

with others of its kind from a lot of univoltin eggs received from Padua, Italy, through Professor Kellogg of Stanford University from the director of the Royal Silk-culture Station. The male parent was a white cocooner from an unbanded whitish but strongly patterned larva of the Japanese bivoltin race. At the time the mating was made, the male was selected for its larval and cocoon character and its bivoltin character was unknown. This was learned later through Prof. Woodworth of the University of California from whom the cocoons had been obtained.

The 230 larvæ reared in the spring of 1905 from this 1904 cross were all banded and all strongly patterned. Thus the color character of the brood partook in toto of the color pattern of each parent. The cocoons spun by these larvæ were neither salmon (the female cocoon color) nor white (the male cocoon color) but a grade between the two. They were a shade of light yellow that might be termed a blend. For the most part the color was uniform, but in several (comparatively few) individuals it approached more nearly to that of the female parent.

These cocoons are now deposited, with cocoons of parental broods, in the museum of the Department of Entomology and Bionomics of Stanford University.

We can say therefore of this first cross that neither the larval "color and pattern" character nor cocoon color of either parent is exclusively dominant in the first generation. The characters may be said to have equal potency in this generation.

The brood however as a whole followed the racial character of its female parent in regard to its voltinism (if I may use this term to express the number of generations destined to be produced in a year). That is, the brood was univoltin in the first gen ration (1904). I will designate this first generation brood as hybrid uni(bi)voltin, indicating that univoltinism is expressed and bivoltinism is not expressed. In later broads where bivoltinism is expressed and univoltinism not expressed, the broods will be referred to as hybrid bi(uni)voltin.

From this isolated cross one could not say whether this was a case of "dominance" of univoltinism in a Mendelian sense, prepotency of the female parent or prepotency of the univoltin character as such. The later history of this lineage throws much light on this point.

It so happened, somewhat by accident and somewhat by design, that there were 34 matings made in the spring of 1905, (out of the total number of 210 matings) in which one or both parents were taken from this brood.

These matings were distributed as follows:

Series A 24 matings of univoltin \$\varphi\$ (pure) \times hybrid uni(bi)-voltin \$\delta\$

Series B 5 matings of hybrid uni(bi)voltin  $\mathcal{P} \times \text{hybrid}$  uni-(bi)voltin  $\mathcal{P}$ 

Series C 5 matings of hybrid uni(bi)voltin  $\circ$  × univoltin  $\circ$  (pure).

Full lots of eggs were obtained from each mating, namely, 34 masses of eggs. In June about 15 days after the eggs were oviposited, when a close inspection of the eggs was made, all of the eggs in Series A had assumed the gray tone of the univoltin egg. Of the five broods of eggs secured in Series B, two broods had assumed the gray appearance of the univoltin egg, while three broods failed to go into the univoltin condition but hatched at this time. Of the five broods of Series C, as in the previous series, two broods assumed the gray appearance of the univoltin egg while three broods hatched at this time.

The results in these three series may be tabulated thus:

TABLE I

(A dash (—) in the second column indicates univoltin or failure to bivolt in the year designated. In succeeding tables, in the same column, plus sign (+) indicates bivoltin in the year designated)

BROO	D CONDI	TION OF FARENTS	TOTAL NUMBER OF BROODS	NUMBER OF BIVOLTS	NUMBER OF UNIVOLTS	PER CENT OF UNI- VOLTS 1905
Series A	-04	U Q × Hy U (B)&	24	0	24	100
Series B	-04	$HyU(B) \circ \times HyU(B) d$	5	3	2	45
Series C	-04	Hy U (B) Q X U&	5	. 3	2	40

Thus it is that in the first generation, through a bivoltin male (and pure univoltin female), bivoltinism failed of expression and in the second generation through a hybrid uni(bi)voltin male (and pure univoltin female), (Series A), bivoltinism again failed of expression; while in the second generation through a hybrid uni(bi)voltin female, bivoltinism reappeared through some mothers and failed through others whether or not these had hybrid mates (Series B and C). It seems also that there is an equal chance for the reappearance of the bivoltin condition through the female uni(bi)hybrid whether she has a uni(bi)hybrid mate or a pure univoltin mate. In other words, the condition of the brood with reference to the character in question is due to the condition or potency of the mother regardless of the condition of her mate, but inherited it may be through her male ancestor and may therefore be interpreted as a purely maternal though racial character.

The question now arises, is the race-character of the 1904 male parent lost completely from the lineage of the 24 male offspring forming the male parents in Series A and from the four broods in Series B and C, or is it simply rendered inactive. Further results show the latter to be the case.

The six broods of Series B and C, bivolting in 1905, were reared in the summer of 1905 and many matings made for the spring rearing of 1006. During a certain exigency in the spring of 1006, all but 12 broods, the offspring of broods in Series B, were abandoned. These 12 broods with 12 broods from the spring lot of 1925 that failed to bivolt (Series A), were reared in the spring of 1906. Thirty matings were made in each of these series. Series D is made up of matings within the bivoltins of Series B, i. e., spring matings of moths from the summer bivoltins of 1905, whose parents were hybrid uni(bi)voltin, that had failed to bivolt in 1904, (hence - 04 +05). Series A' is made up of matings of moths within 1906 broods, that had failed to bivolt in 1905 whose male parent was a hybrid uni(bi)voltin and whose female parent was a pure univoltin, (hence -04 -05). Of the 30 matings in Series D, four broods hatched in the summer of 1906, that is, were bivoltins, the rest of the series having entered into univoltin condition. A ratio of 6.5 U: 1 B or 86 per cent

of univolts therefore obtained. Of the 30 matings in series A', two broods hatched or were bivoltin in 1906. A ratio of 14 U:1 B or 90 per cent of univolts obtained.

For convenience of reference, these results may be tabulated thus. (See also "Table of Descent.")

Name of the American Particular Control	BROOD CONDITIONS OF PARENTS AND LINEAGE	TOTAL NUMBER OF BROODS	NUMBER OF	NUMBER OF UNIVOLTS	RATIO OF B: U (1906)	PER CENT OF U 1906
Series A'	-04 -05	30	, 2	28	1:14	90+
Series D	-04 +05	30	4	26	1:6.5	86+

TABLE II

Three points present themselves for consideration from a study of Tables I and II; first, relative potency of the bivoltin and univoltin in Series A' and Series D, Table II; second, relative potency of these characters in Series B, Table I, and Series D, Table II; third, suppression of the bivoltin in Series A, and its expression in Series A.'

In regard to the first point; namely, relative potency as correlated with lineage in the expression of the characters, the data show that the larger percent ge of univolts obtains in the line of the greater suppression of bivolts. With reference to the second point, the small number of matings in Series B may account for the seeming prepotency of the bivoltin in 1905 (Table I) as against its subpotency in 1906, Series D, (Table II). It may be, however, that this prepotency obtains in first crosses of the hybrid uni-bivoltin cross through the female. This point will be further tested. The fact with reference to the third point is certain. Failure of the bivoltin character for two successive generations through the univoltin female (with a bivoltin male in 1904 and a hybrid uni(bi)voltin male in 1905) does not preclude the possibility of its reappearance in the third generation (1906).

It was determined at this time to utilize this material to its utmost advantage for a further study of heredity.

It is recognized that for absolutely adequate results, four large series of matings should have been made in 1906, that is, about 200 matings of bivolts in Series A' with a like number of matings of univolts in the same series, the same number of matings of bivolts in Series D and a like number of matings of univolts in the same series.

The arduous labor connected with the breeding of these insects under experimental conditions precludes the possibility of rearing in all series these large lots unless one has undivided time to give to the work and much assistance. It was therefore determined as the only practical thing to do, to rear one series from year to year in large numbers and to reduce the other lots to a minimum. It is hoped that the results thus obtained may add somewhat to our knowledge of "laws of inheritance."

In pursuance of the above plan, a total of three hundred and sixty matings were made in the spring of 1907 in the following three series.

Series E, the 1906 univoltin material that failed to bivolt in Series A'(-1905, -1906), 316 matings.

Series F, the 1906 univoltin material of Series D, (+1905, -1006), 21 matings.

Series G, the 1906 bivoltin material of Series D (+1905, +1906), 23 matings.

The distribution and results of these matings may be tabulated as follows (see also "Table of Descent"):

BROOD TOTAL RATIO OF PERCENTAGE CONDITIONS NUMBER OF NUMBER OF BIVOLTS TO OF UNIVOLTS NUMBER OF UNIVOLTS OF PARENTS BIVOLTS BROODS UNIVOLTS 1907 AND LINEAGE Series E.....-04-05-06 88+ 216 284 1:9-32 S ries F.....-04+05-06 21 1:4+ 80+ 17 Series G.....-04+05+06 16 1:2-69+ Total ...... 360

43

317

TABLE III

Thus in the three series together there were a total of 43 bivoltin broads in 1907. These broads were reared in the summer of 1907.

In the summer of 1907, five series of matings were made within these series and in the spring of 1908, these five series of broods were reared from the eggs that had lain over the winter. These matings were distributed as follows:

Series H, the 1907 bivoltin material of Series E, Table III. Series I, the 1907 bivoltin material of Series F, Table III. Series J, the 1907 bivoltin material of Series G, Table III. Series K, the 1907 univoltin material of Series E, Table III. Series L, the 1907 univoltin material of Series F, Table III.

In the spring of 1908, when these broods had reached maturity, a total of 298 matings were made.

The distribution and results of these matings may be tabulated thus (see also "Table of Descent"):

	BROOD CONDITION OF PARENTS AND LINEAGE	TOTAL NUMBER OF BROODS	NUMBER OF BIVOLTS	NUMBER OF UNIVOLTS	RATIO OF BIVOLTS TO UNIVOLTS	PERCENT- AGE OF UNIVOLTS 1908
Series H	-04-05-06+07	209	31	178	1:5	85+
Series I	-04+05-06+07	31	9	22	1:2	70+
Series J	-04+05+06+07	12	6	6	1:1	50+
Series K	-04-05-06-07	35	9	26	1 : 2 h	74+
Series L	-04+05-06-07	11 '	3	8	2-1:1	73+

TABLE IV\*

Comparing Series A (Table I), Series A' (Table II), Series E (Table III) and Series K (Table IV) representing continuous selection of hybrid uni(bi)voltin through four consecutive years (four generations from the hybrid parent), we find the univoltin continuously predominating but with gradually decreasing percentage; thus, 100 per cent (1905), 90 per cent (1906), 88 per cent (1907) and 74 per cent (1908).

Comparing Series B (Table I), Series D (Table II), Series G

<sup>\*</sup>Note added September 20, 1909. In 1909 Series H, through the bivoltin line produced 74 per cent U; Series I produced 43 per cent U; Series J produced 32 per cent U.

(Table III), and Series J (Table IV), representing continuous selection of the hybrid bi(uni)voltin through four consecutive years (eight generations from the hybrid parent), we find again the univoltin continuously predominating from 1906 and a gradual decrease in the percentage of univolts until in 1908 the potency of the two characters seem to be equalized; thus, 40 per cent (1905), 86 per cent (1906), 69 per cent (1907), 50 per cent (1908).<sup>2</sup>

These two groups of percentages show that there is some potent influence in the bivoltin as a character that counts for its accumulation from year to year whether it is expressed in the parents selected, as in B, D, G, and J, or has not been manifest in these, as in A, A', E and K (see also "Table of Descent").

It is also strikingly noticeable that in the former group where selection was made continuously through the line in which the bivoltin character was expressed, the decrease in the percentage of univolts (increase in percentage of bivolts) is much more rapid (with the exception of the unexplained low percentage of 1905) than in the latter group where selection was through the line in which the character was not expressed.

If we consider a third group of matings, Series D (Table II), Series F (Table III), and Series I or Series L (Table IV), we find the same consistent decrease in the percentage of univoltinism, 86 per cent (1906), 80 per cent (1907), 70 per cent or 73 per cent (1go8).

It may be argued, and the argument might be a valid objection to results in all series except A, A', E and H, that the number of matings made is too small for adequate results. But since in the series of smaller lots, the results are in general consistent with the results obtained in the larger lots we may, I think, consider that on the whole the small size of certain lots has not materially interfered with normal general results. If we base our conclusions on series A, A', E, and K independently, or include all the series in our considerations, the results are the same.

This g adual decrease of the percentage of univoltins seems traceable to one thing. The persistent predominance of the univolt appears to represent the pull or "drag" of univolt ancestry, while its gradual decrease under each condition appears

<sup>\*</sup>And in 1909, 32 per cent, thus becoming sub-potent.

to denote the potency or strength of its opponent bivoltinism. It looks as though by long selection univoltinism had become prepotent but that the possibly older bivoltinism, when once introduced is able gradually to reëstablish itself in the heredity.

The question now arises, Is the condition of individuals taken as separate entities coincident with the condition of the average of the series? In 1907, and again in 1908, precaution was taken to make a number of matings within each brood, to throw light on this query. It was found that all the tested females furnished by a few broods, particularly in 1908, were bivoltin-producing. Many of the broods furnished univoltin-producing females only and others furnished females part of whom were bivoltin-producing and part of whom were univoltin-producing. Of the latter group, sometimes univoltin producers predominated and sometimes bivoltin producers predominated. In each case these females were mated with males of similar ancestry. This diversity of behavior is in general true not of any one series alone, but of all series.

For exact and graphic comparison, I have selected eight broods in Series E of 1907 and ten broods in Series H, I and J of 1908, from each of which ten mothers were chosen as parents. Diagram I shows the comparative number of univoltin and bivoltin broods in the former group (Series E). In this diagram the vertical distance in each case indicates the number of bivoltin-producing mothers (out of the total of ten mothers) furnished by each of the eight broods respectively:

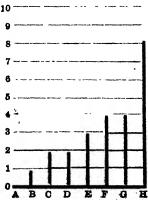


Diagram 1. Showing distribution of univoltin- and bivoltin-producing mothers in each of eight broads

Thus, Brood A furnished no bivoltin-producing but ten univolutin-producing mothers; Brood B furnished one bivoltin-producing and nine univoltin-producing mothers; Broods C and D each furnished two bivoltin- and eight univoltin-producing mothers; Brood E furnished three bivoltin-producing and seven univoltin-producing mothers; Broods F and G furnished four bivoltin-producing and six univoltin-producing mothers; Brood H furnished eight bivoltin-producing and two univoltin-producing mothers. The maximum number of bivoltin-producing mothers from a single brood in this group is eight, and from this the number grades down to zero. And yet, as previously stated, in Series E, Table III, in this series as a whole, the ratio of univolts to bivolts is as 9:1. This high proportion of univolts comes from the fact that there were many more broods furnishing univolts only, than bivolts, taking the series throughout as a whole.

Diagram 2 shows the comparative number of univoltin and bivoltin broods in the second group of broods from which ten individuals were selected as mothers (Series H, I and J).

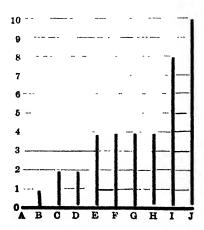


Diagram 2. Showing distribution of univoltin- and bivoltin-producing mothers in each of eight broods

Thus Brood A furnished no bivoltin-producing but ten univoltin-producing mothers; Brood B furnished one bivoltin-producing and nine univoltin-producing mothers; Broods C and D each furnished two bivoltin-producing and eight univoltin-producing mothers; Broods E, F, G and H each furnished four bivoltinand six univoltin-producing mothers; Brood I furnished eight bivoltin and two univoltin-producing mothers and Brood J furnished ten bivoltin- and no univoltin-producing mothers.

Thus it is that the averages of the various series as wholes do not represent the actual brood condition of each brood within the series. Certain broods are totally prepotent in the direction of the expression of univoltinism, certain broods are totally prepotent in the direction of the expression of bivoltinism. The whole study indeed points to an underlying "law of potency." Such a law has been suggested by Davenport, but as yet the study of heredity is too young for any adequate expression of the law or any comprehension of its real significance.

Two years after this study was begun K. Toyama published a paper on "Some Silkworm crosses with special reference to Mendel's Law of Heredity."4 The main part of his paper is devoted to a consideration of crosses involving larval and cocoon charac-However, he devotes one page to a consideration of a cross similar to that of the present study. In Toyama's report, of five univoltin + bivoltin crosses, the eggs laid by the moth were, in four cases, like the maternal race,—that is, bivoltin when the mother was bivoltin (2 cases) and univoltin when the mother was univoltin (2 cases). In one cross, the eggs followed the paternal race character. Toyama adds a footnote reference to the effect that "further experiments show that the first cross is always maternal in crossing with pure breeds." It will be remembered that in the original cross of the present work (1904), the first generation followed the character of the maternal race, as in Toyama's four crosses; namely, was univoltin from a univoltin female and a bivoltin male.

In the spring of 1908, after it was determined that certain broods reared from 1907 parents were furnishing bivoltin-producing mothers only, and others univoltin-producing mothers only, when mated with males similarly reared (whether or not from identical broods), it was determined to test these strains with

<sup>&</sup>lt;sup>8</sup> Davenport: Heredity and Mendel's Law. Proc. of the Wash. Acad. of Sciences, ix, 1907, p. 184.

Bulletia of the College of Agriculture, Tokyo Imperial Univ., vol. vii, 259-293.

pure alternates. Accordingly, ten females, each from a brood that had proven its bivoltin-producing capacity, were mated with males of pure univoltin extraction. This is practicable because of the great difference in time of the issuance of moths from cocoons of the same brood. The eggs of each of these ten moths were bivoltin or maternal.

There were also 35 matings made between pure univoltin females and males from broods that were known to be furnishing bivoltin-producing females. In this case also the eggs were all maternal or univoltin.

Since all the sisters of known bivoltin-producers, produced bivoltins only, with pure univoltin mates, and since no pure univoltin female produced other than a univoltin brood even with a bivoltin mate, it is apparent that it is the condition of the gametes of the immediate female that determines the character of the brood, these gametes embodying a heritage from both grandparents. The character of the race of the immediate male mate may be transmitted but is not expressed.

This lends support to Cook's argument<sup>5</sup> that the conjugation of gametes is not complete at the time of fertilization, complete fertilization taking place only when the "chromatin is fused in mitapsis, preceding the formation of cells for the next generation." It is therefore this generation ("pergugate" of Cook) that will show the full effects of the previous fertilization, or "inheritance-relations" of the characters of the original parental organisms in Cook's view.

While with this particular group of characters there is a failure of expression in each first generation (conjugate generation of Cook) this is not the case with larval nor cocoon color characters as previously noted. Here, plainly as pointed out, the first or conjugate generation partakes strongly of the nature of both parents. Therefore it is, that expression or non-expression of character in the first generation must involve something aside from the fact that fertilization and conjugation as looked upon by Cook are separate entities.

<sup>&</sup>lt;sup>6</sup> Cook, O. F.: Mendelianism and other methods of descent. Proc. Wash. Acad. of Sci., ix, 1907, p. 192.

Certainly, however, with the characters under present consideration it is not until the second generation at least that the results of the cross univolt + bivolt are brought to full expression. However, as indicated in Series K (Table IV) it may take four generations. While the zygotes in Series A (Table I) are predisposed to the expression of the character of the univoltin race, the reappearance of bivoltinism through the univoltin line in successive series (Series A', Series E, Table III and Series K, Table IV) to a very appreciable degree, shows that expression is in no wise du to absence of the alternative or bivoltin character. This character is, on the contrary, simply held in abeyance and transmitted from generation to generation through the germ cells without expression. Hence transmission of this character has no relation to expression or as Cook puts it "the failure of a character to secure expression does not indicate that it has failed of transmission." They are "independent and separate processes."

This brings us to a consideration of potency. To use Cook's synonym "characters which secure stronger or more general expression in the new gametes are called *prepotent*, those which tend to decline or disappear may be called *sub-potent*, and characters which secure an unmodified representation in behalf of the gametes may be called *equipotent*.

From a consideration of the data at hand, it is evident that potency is a phenomenon of a fluctuating nature. Univoltinism is at first, and for a series of years prepotent, becoming less and less so as the generations advance whether or not bivoltinism has found expression in the interim—note Series E (Table III),—88 per cent, and Series K (Table IV), 74 per cent; Series G, Table III, 69 per cent, and Series J, Table IV, 50 per cent. Or what is more striking, compare the various broods in Diagram 2. In Brood A, univoltinism is totally prepotent, in Broods B, C, D, E, F, G, and H, it is partially prepotent and bivoltinism is subpotent; in Brood I, bivoltinism is partially prepotent and

Loc. cit., p. 259.

Note added September 20, 1909. The bivoltins of this series were reared in the spring of 1909. Thirty-four matings were then made within the series, which resulted in 67% Bi. and 32% U. Thus the bivoltin in this series has passed over from a sub-potent to a pre-potent condition.

univoltinism is subpotent, while in Brood J, bivoltinism is totally prepotent. Whether the bivoltin will remain prepotent in the offspring of Brood J and univoltinism will remain prepotent in the offspring of A is yet to be determined.

#### DISCUSSION OF RESULTS

The contrasted characters that are made the basis of this series of experiments are "univoltin-producing" and bivoltin-producing." These characters are known to silk-worm breeders as racial characters. The univoltin race breeds true in pure lots, with the exception of an occasional sport-bivoltin as previously noted. Unfortunately I was unable to obtain any pure bivoltin silkworms except the single individual with which the experiment began. The first generation of the cross U P X B & follows the racial character of the female parent. If the character that fails in appearance in the first generation (the bivoltin) be considered the "recessive" in Mendelian terms, then in the second generation (in matings within hybrids) there should be, according to Mendelian hypothesis, a ratio of three univoltin broods to one bivoltin brood and thereafter the bivolts should breed true. While. in the present instance, the first generation brood follows but one of the divergent parent-characters (univolt when the female is univolt, bivolt when the female is bivolt) in the second generation, the broads fail to follow both parents in the expected proportions. Therefore the Mendelian law does not hold in this case.

If we consider that the phenomenon may be Mendelian complicated by a distinct correlation between sex and character, we still must account for the abeyance of the character for several generations and its later reappearance in certain strains. If this can be accounted for through the successive failure of the meeting of the right "determiners" then, according to Mendelian expectation we must look for the "breeding-true" of the reverting individuals. This is true only in a very limited number of broods and only toward the end of the series.

On the contrary, this case seems to add to the evidence adduced

by Castle, Davenport, Weber, and other thrematologists that the Mendelian conception of purity is at least not applicable to all classes of characters. It shows as Webber has shown in a later paper on "Plant morphology" (quoted in paper cited), that the anlage of a character, in the present instance "bivoltinism" may be masked for several years and then reappear to an appreciable degree.

The evidence points strongly to a difference in female and male potency in ability to give expression to the character, the difference in potency of transmission being mainly (if not entirely) dependent upon the nature of the characters concerned and their relative potency, this being presumably determined by their past history. The evidence shows conclusively that while though failure of expression occurs in the first, second and even third generation, the non-expressed character must be reckoned with as part of the inheritance and its reappearance looked for.

It shows further that the character bivoltinism loses none of its potency although not exhibiting any outward manifestation, but, on the other hand gains or is reinforced through reversions.

While the process of selection may be materially assisted by selecting prepotent individuals, ignoring these does not preclude the possibility of effective selection. This depends upon the relative value of the characters concerned. The relative value appears to change from generation to generation according to the amount of each character invoked in the cross modified by the normal relative potencies of each. For instance, bivoltinism begins in Series A' (Table II) with a percentage value of less than 10 per cent. It accumulates from generation to generation, through lines in which there is no "expression" until, in Series K (Table IV) it shows a percentage value of over 25 per cent. In Series D (Table II) we find it with a percentage value of about 14 per cent. It accumulates from generation to generation through lines in which there is "successive expression" until in

<sup>&</sup>lt;sup>8</sup> Castle: Origin of a Polydactylous Race of Guinea Pigs. Contri. from Zoöl. Lab. of Mus. of Comp. Zoöl. of Harvard College, no. 176, 1906, p. 29. Pub. of Carnegie Inst. of Washington, no. 49.

<sup>8</sup> Loc. cit., 1907.

<sup>18</sup> Weber: Some Gaps in our Knowledge of Heredity. American Breeders' Association, iii, p. 344, 1908.

Series J, (Table IV) it shows a percentage value of 50 per cent<sup>11</sup> (see "Table of Descent').

#### SUMMARY

An account is given of a series of breeding experiments extending throughout five years, from the spring of 1904 through the summer of 1908. These experiments originated in a single cross of 1904 involving a female univoltin silkworm and a male pure bivoltin silkworm.

The essential feature of the breeding is that matings were confined within series representing certain conditions of ancestry so that each lineage could be traced back to the original cross and the different lineages compared. It is pointed out that each character is transmitted by either parent but finds expression through the female parent only. It is further pointed out that lack of expression of the characters in question is no indication that they have failed in transmission, that is, "transmission of a character has no relation to its expression."

It is noted that there is no uniform proportion such as is observed in Mend lian crosses between the characters in first and succeeding generations. On the contrary there is a noticeable fluctuation of prepotency from one character to the other as generations ensue. The univoltin is at first prepotent. As the hybrid uni(bi) oltin generations increase the percentage of univoltins decreases. As the hybrid bi (uni)voltin generations increase the percentage of univoltins also decreases and much more rapidly.

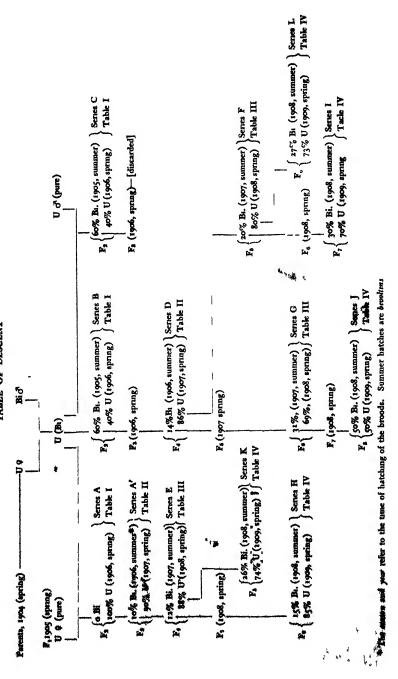
It is suggested that the past history of the characters probably has much to do with their present interrelation, that is, that the younger univoltin, though long selected cannot hold its own against the older bivoltin although it exhibits a perceptible pull for several generations.

It seems that as between these intra-specific characters, there is an underlying "law of potency" that has to do with characters as ancestral rather than with characters as parental units.

<sup>11</sup> Note added September 20, 1909. In the summer of 1909 bivoltinism shows a percentage value of 67 per cent in this series.

Bionomics Laboratory Stanford University, California June, 1909

# TABLE OF DESCRIT



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